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The ecology, distribution, status, threats, and conservation of the Common Water Monitor (*Varanus salvator*) in the Dholeswari River of Assam, India

^{1,*}Muhammed Khairujjaman Mazumder, ²Amir Sohail Choudhury, ²Rofik Ahmed Barbhuiya, ³Himabrata Chakravarty, and ⁴Badruzzaman Barbhuiya

¹Department of Zoology, Dhemaji College, Dhemaji, 787057, Assam, INDIA ²Department of Ecology and Environmental Science, Assam University, Silchar 788011, Assam, INDIA ³Department of Zoology, Srikishan Sarda College, Hailakandi 788151, Assam, INDIA ⁴District Level Laboratory, Public Health Engineering Department, Hailakandi 788155, Assam, INDIA

Abstract.—The Common Water Monitor, *Varanus salvator* (Laurenti, 1768), is a large monitor lizard distributed in southern and south-east Asia, including India which remains closely associated with water bodies, such as rivers and lakes. Although IUCN considers it to be ‘Least Concern,’ the Common Monitor Lizard faces several threats throughout its global distribution range, and the status of the species is decreasing rapidly. The Dholeswari River of Assam (India) is one of the most important abodes of this species, where it is locally known by the names ‘Irong’ and ‘Shanda.’ Geographically, the Dholeswari River is located in southern Assam (India), which falls within the Indo-Burma Biodiversity hotspot area. Unfortunately, most of the wildlife of southern Assam (India) are poorly studied, and this varanid is one of the most ignored species of the region. The present study was conducted along the Dholeswari River, Assam, India, to elucidate the distribution, status, ecology, threats, and conservation of the Common Water Monitor, and is the first report on this species from this river. The results show that the Dholeswari River still serves as a habitat of the species, with a viable population. Further, the species was found to prefer smaller rivers with clayed soil and bushes, and it faces major threats from habitat destruction, hunting for flesh and oil, and conflicts with humans. Based on our observations, we discuss recommendations for the conservation of this large varanid.

Keywords. Asia, Barak Valley, Indo-Burma Biodiversity hotspot, Reptilia, Sauria, Varanidae

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Introduction

The Common Water Monitor (*Varanus salvator*) is the largest monitor lizard of India, and second largest of the world, after the Komodo Dragon (*V. komodoensis*). It has the widest global distribution among all varanids (Traeholt 1994). The distribution of the species in the Indian subcontinent was described by Das (1994), Daniel (1983), and Koch et al. (2013), while Smith (1935) reported its occurrence and distribution in Northeast India, of which the state of Assam is a part. Anderson (1982) and Auffenberg (1986) reported the occurrence of this species from Assam (India). Among the four recognized subspecies of the Common Water Monitor, *V. s. macromaculatus* occurs in Assam (Traeholt 1994; Auffenberg 1986). The Common Water Monitor is generally found associated with water systems including

rivers and wetlands (Ahmed et al. 2009; Cota et al. 2009). It is seldom found far from water (Smith 1935), and only rarely beyond 200 m from water bodies (Cota et al. 2009). The Common Water Monitor has a wide trophic niche, and it consumes a variety of prey species including crabs, fishes, other lizards, snakes, domestic fowl, and eggs of other animals, in addition to scavenging (Ahmed et al. 2009).

Some of the important publications on the reptiles of Northeast India include Ahmed et al. (2009), Das (2008), and Choudhury (1989, 1992, 1993a,b, 1995, 1996a,b, 1998, 2011). However, none of these articles provide special treatment of either the Common Water Monitor, or southern Assam. Southern Assam (India), is also known as the Barak Valley, and comprises the districts of Cachar, Hailakandi, and Karimganj. It is a part of the Indo-Burma Biodiversity hotspot, and

Correspondence. *khairujjaman1987@gmail.com

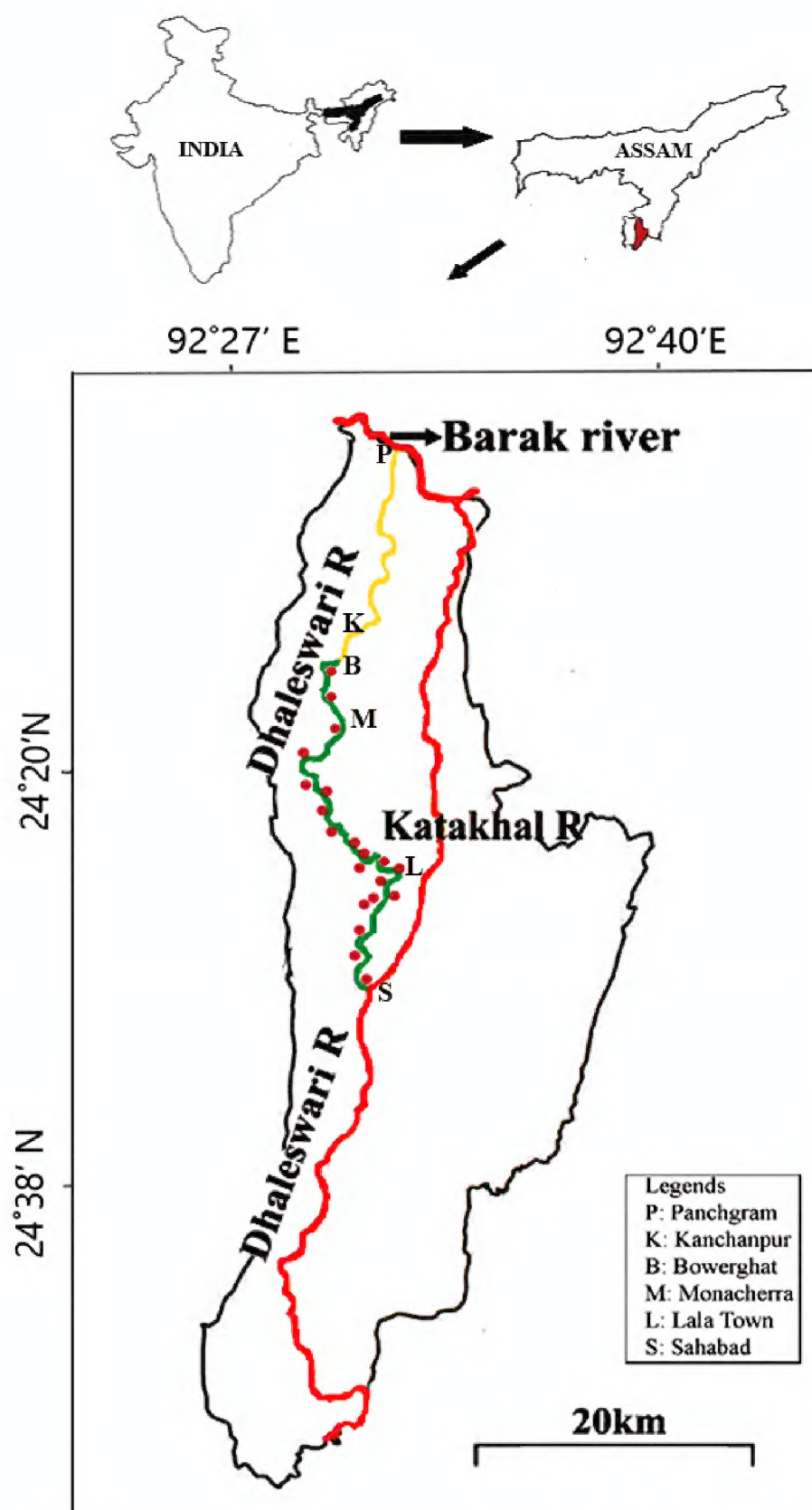


Fig. 1. (A) Map of India, highlighting Assam. (B) Map of Assam, highlighting Hailakandi district. (C) Map of Hailakandi district showing the two rivers, Dhaleswari River and Katakhal River, with distribution of the Common Water Monitor (*Varanus salvator*). Dots represent sighting locations during the present survey; current distribution in the Dhaleswari River is shown in green. Further downstream, despite no present records, occurrence in the past (1970s–1980s) was reported by several interviewees (shown in yellow). No reports on present occurrence in the Katakhal River (shown in red) could be found. Map by A.S. Choudhury.

harbors a myriad wildlife assemblage (Myers et al. 2000; Choudhury 1997, 2013; Mazumder 2014), including a diverse herpetofauna (Ahmed et al. 2009). Two species of monitor lizard are reported to inhabit the region, the Bengal Monitor (*Varanus bengalensis*) [Das 2008] and the Common Water Monitor (*Varanus salvator*) [Ahmed et al. 2009; Whitaker and Whitaker 1980]. Once common in most of the rivers of Assam, the habitat of the Common Water Monitor is decreasing rapidly (Ahmed et al. 2009), although its IUCN status currently remains Least Concern (Bennet et al. 2010).

The present study was conducted in the Dhaleswari River of southern Assam (India) to elucidate the distribution (past and current), status, ecology (behavior and habitat), threats, and conservation of the Common Water Monitor. Based on the findings of this study, we provide recommendations for the conservation of this species. The present study is particularly important since some of the majestic mega-fauna of southern Assam, including Gaur (*Bos gaurus*), Asiatic Wild Water Buffalo (*Bubalus arnee*), Indian One-horned Rhino (*Rhinoceros unicornis*), Sumatran Rhino (*Dicerorhinus sumatrensis*), Javan Rhino (*R. sondaicus*), Royal Bengal Tiger (*Panthera tigris*), and notably two mega-reptiles: the Gharial (*Gavialis gangeticus*) and Marsh Crocodile (*Crocodylus palustris*), have been extirpated in the last century (Choudhury 1997, 1998, 2013, 2016; Singha 2009), while the Ganges River Dolphin (*Platanista gangetica gangetica*) is on the brink of extirpation (Mazumder et al. 2014).

Materials and Methods

Study site. The present study was conducted in the Dhaleswari River, located in the district of Hailakandi, in southern Assam of India. The climate is of the tropical monsoon type, with average elevation of 21 m asl, average annual precipitation of 2,400–2,800 mm, and the temperature varies from 37 °C in the summer to 7 °C in the winter (Choudhury and Choudhury 2017). The Dhaleswari River originates in the Mizo Hills in the state Mizoram (India), flows through hilly terrains towards north, and enters the Hailakandi district. Further downstream, the river bifurcates at Shahabad (Gainja-Khauri) of Hailakandi (24°28'51.8"N, 92°34'38.3"E), whereby its water is diverted to an artificial channel now called Katakhal River with a sluice gate, thereby eliminating the immediate downstream section. However, further downstream, smaller streams join the river, thereby enhancing its flow discharge, and it finally drains into the Barak River at Panchgram, Hailakandi (24°51'54"N, 92°36'32"E; Fig. 1). The length of the river from Shahabad to Panchgram is approximately 110 km, with an elevation ranging from 20–33 m. The river is almost dry with no flow in the winter season, especially in the upstream half of its length, while significant flow is attained in the monsoon season (April to October).

The present study was conducted in a stretch of the river from Shahabad (24°28'51.8"N, 92°34'38.3"E) to Bowerghat (24°38'37.4"N, 92°32'13"E), covering an approximate length of 56 km. The area is inhabited by Bengali community people who cultivate rice paddies (between June and November) in the plain areas, and vegetables (between November and March) in the plains as well as on the river banks. The river bank has an abundance of bushes and bamboo groves, as well as large trees. Brief surveys were also made in the Katakhal River (the other river of Hailakandi district) from Sahabad to

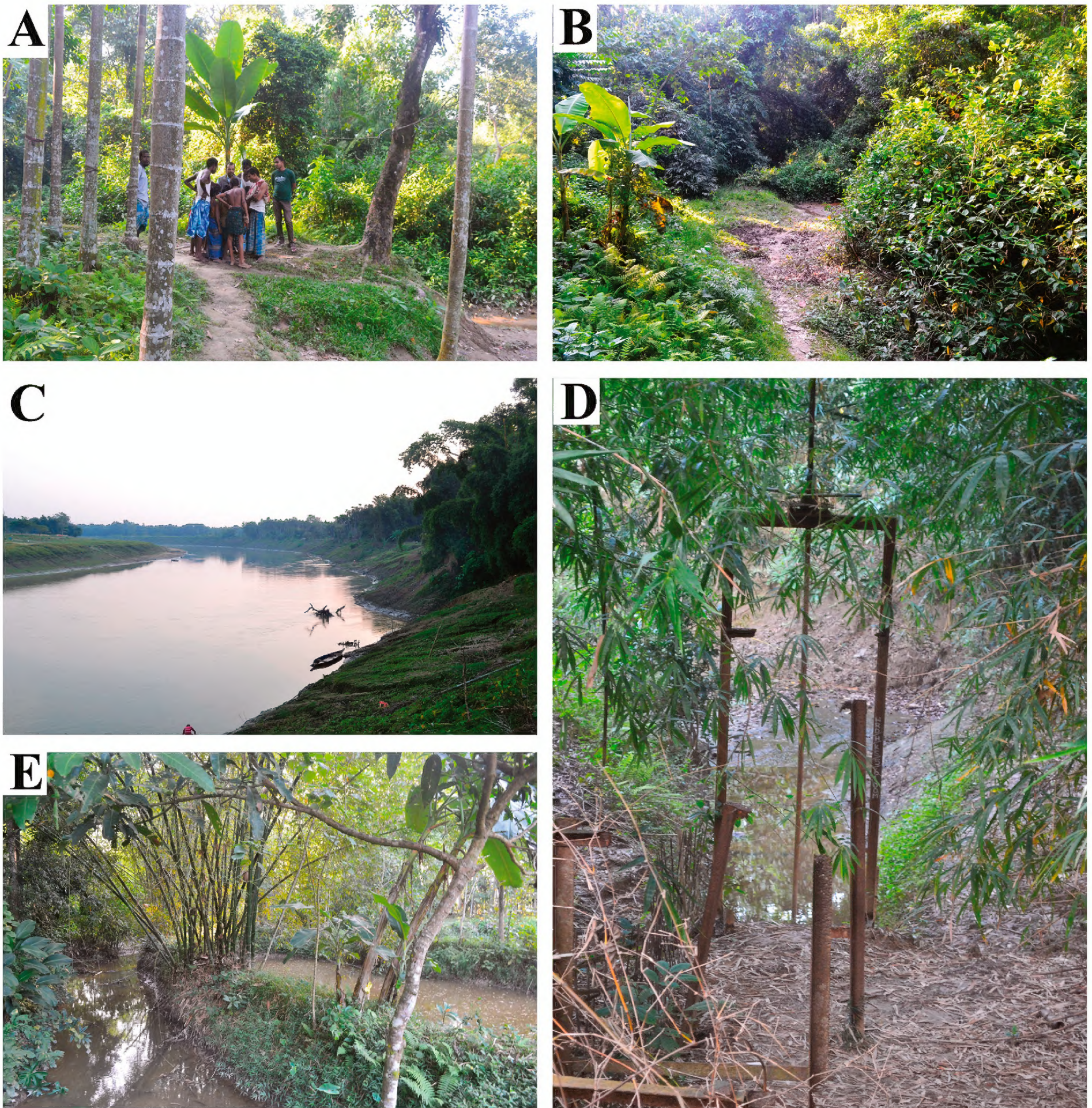


Fig. 2. Photographs relevant to the habitat and threats of the Common Water Monitor (*Varanus salvator*) in the Dhaleswari River, Assam, India. (A) Research team interacting with the locals at Rongpur 5, Hailakandi. (B) Habitat of the Common Water Monitor in the Dhaleswari River, showing bushes and other features on the banks. (C) The sandy bank of the Katakhal River, prone to erosion and landslides, is the habitat not preferred by the Common Water Monitor. (D) The sluice gate at the mouth of Dhaleswari River at Shahabad, which prevents water flow into it and diverts the water to the Katakhal River. (E) Encroachment and conversion of the Dhaleswari River into fisheries by the locals building dikes at Rongpur 2. Photos by A.S. Choudhury.

its confluence with the Barak River at Katakhal Mukh (24°51'21"N, 92°37'27"E; Fig. 1). The Katakhal River has greater depth, width, flow rate, and flow discharge, and it has abrupt banks (with sandy soil) which experience frequent and severe erosion and landslides.

Survey techniques. Preliminary discussions with the riparian local people and forest officials were conducted to locate extant populations in the Dhaleswari and Katakhal rivers of Hailakandi, southern Assam. The discussions revealed that the Common Water Monitor

does not occur in the Katakhal River, while it continues to inhabit the Dhaleswari River between Shahabad and Bowerghat (Fig. 1). Two researchers independently carried out an on-foot survey simultaneously along both banks of the Dhaleswari River, covering a length of 56 km (of the total river length of ~110 km), from Shahabad to Bowerghat, to conduct focal counts of the species and the number of nests available in the river bank. The burrows made by the lizard were considered to be nests, while others, including crevices and burrows of other animals, were considered temporary refuges. The

Table 1. Geo-coordinates of locations along the Dhaleswari River, Assam, India, where live specimens of the Common Water Monitor (*Varanus salvator*) were sighted during the survey, including the number of individuals sighted at each location.

Site No.	Location	Geo-coordinates		No. of individuals sighted
		Latitude	Longitude	
1	Shahbad	24°28'51.8"N	92°34'38.3"E	1
2	Shahabad 1	24°29'31"N	92°34'33"E	1
3	Rongpur 2	24°30'18.4"N	92°34'39.8"E	1
4	Rongpur 5	24°31'31"N	92°34'36"E	1
5	Rongpur 5	24°31'39.7"N	92°35'00.7"E	2
6	Rongpur 4	24°31'42.6"N	92°34'57.7"E	2
7	Abdullahpur 1	24°32'08"N	92°35'23"E	2
8	Tantoo Road Bridge	24°32'22.1"N	92°35'33.2"E	1
9	Dhanipur	24°33'29"N	92°35'48"E	2
10	Lala Rural College	24°33'32"N	92°36'12"E	2
11	Lalaghat Nala confluence	24°33'36"N	92°35'21"E	3
12	Kaya Khal confluence	24°34'00"N	92°34'24"E	2
13	Bhabanipur	24°34'19"N	92°34'54"E	2
14	Sarbanandapur	24°34'19"N	92°34'51"E	1
15	Behula	24°34'44"N	92°34'03"E	1
16	Aenakhil Tea Factory	24°35'18"N	92°33'04"E	1
17	Aenakhil Market	24°35'55"N	92°32'52"E	1
18	Monacherra (Lakhinagar)	24°36'45"N	92°32'54"E	3
19	Kukinagar	24°37'26"N	92°32'22"E	2
20	Bowerghat	24°38'37.4"N	92°32'13"E	1

diameter of each nest hole was measured and topology was identified whenever possible. Different types of nests were recorded, which were verified by local guides. Upon sighting of a lizard, the Global Positioning System (GPS) coordinates were recorded using a digital GPS machine (eTrex 20X, Garmin, China). The surveyors made keen observations in the trees for possible occurrences of the varanid, since this species has been reported to climb trees for basking (Ahmed et al. 2009). Since no specimens were found in the Dhaleswari River further downstream of Bowerghat, or in the Katakhal River, those negative data are not incorporated into the further analysis. The total time spent in the field survey, for direct observations and counting of the lizards, was 118 h for each observer. In addition, potential threats to the monitor lizard and its habitat were observed during the survey.

Interviews of local people. Interviews of the locals ($n = 30$), generally the older folks, were conducted regarding the occurrence of the lizards, their nesting places, food, incidences of hunting, and potential conflicts (Fig. 2A). In addition to the local people of the area, many others hailing from different localities in southern Assam were also interviewed to get an idea of the past and present occurrences of the monitor lizard in the Hailakandi district, and in the Dhaleswari River and Katakhal River in particular. The interviews used colored photographs, pictorial guides and sketches, and

a closed-ended questionnaire. The total time spent on the interviews was 55 h.

Results

Distribution and Population Status

Past and current distribution. Interactions with local people, forest personnel, and elderly people of the villages revealed that in the 1970s, the whole length of the Dhaleswari River (~110 km) had viable and abundant populations of the Common Water Monitor. However, the population declined gradually, and the species currently inhabits only the section of the river between Shahabad and Bowerghat (Fig. 1). The species also occurs in the tributaries or smaller water channels (*nullahs*) joining the Dhaleswari River in this river section, most importantly the Lalaghat Nala, Kaya Khal, and others. In addition, stray individuals have been reported from Kanchanpur (~10 km downstream of the current range). It is often found near human habitations, crop fields, ponds, and lakes near the current main distribution range. Neither interviews nor surveys revealed the occurrence of this species in the Katakhal River, and we therefore argue that the species has been extirpated from this river.

Population status. During the study, 32 individuals were directly sighted at 20 locations in the 56 km of the river

section surveyed, for a linear density of 0.57 individuals/km of the river course (Table 1). The total number of active nests found during the survey was 215, for a linear density of 3.84 nests/km of the river section. In addition, the survey found 63 burrows and crevices which were used by the lizards as refuges.

Ecology

Habitat and nesting ecology. The current habitat of the monitor in the Dhaleswari River includes areas with river channel widths of 1–5 m in the winter months, with no water present. However, in the monsoon season, the river width may reach up to 30 m, with significant flow and discharge. The depth of the river in the study area varies from 0–10 m. The width of the river bank is 10–30 m, and the banks become flooded with the high waters of the monsoons. The river banks are inclined, and not vertically abrupt. The approximate distances of human habitations from the river bank range from 5–50 m. The river bank is characterized by the presence of bushes, secondary tree growth, planted woodlands, bamboo groves, and occasional crop fields (mainly vegetables), with or without bamboo fencing (Fig. 2B). However, the lizard is absent in the Katakhal River, which indicates that it does not prefer a river where flow and discharge are high, and banks are abrupt and steep with sandy soil which are prone to erosion (Fig. 2C).

The lizard made nests in the inclined river banks in areas where anthropogenic pressure is less intensive. The diameters of the nest entrance holes were 20–25 cm (23.59 ± 1.14 cm; mean $\pm$ SD; $n = 40$). The vicinity of the nests was very clean, due to movement of the lizards, while the entries of some nests were half-sealed with soil. The entries of the nests were horizontal, or inclined downward or upward; however, the tunnel rose upwards immediately at an approximate distance of ~ 0.3 – 0.6 m from the entrance hole. Several other micro-habitats were found, which were natural crevices or burrows of other animals that were used by the monitors, probably as refuges. In these larger crevices, the lizards generally occurred in groups of several individuals.

Behavioral ecology. During winter months, the Common Water Monitor basks in the sun, and has been found to climb trees for basking, especially in the morning. During monsoons, the river water level increased and the nests were flooded. This factor drove the monitors to hide in the bushes, forage near human settlements, and shelter in trees. According to the locals ($n = 30$), the monitor has been found to feed on fishes, snakes, small lizards, crabs, and carcasses of other larger animals which were thrown into the river. There are several poultry farms near the river banks, as well as slaughter houses near populated areas, and people often dispose of carcasses and remnants of slaughtered animals in the rivers. In such places, the Common Water Monitor was frequently encountered.

Monitors with newborns were observed in the month of April, suggesting that eggs hatched in the early monsoon showers which coincided with the nests becoming submerged by flood waters. Also, the abundance of fish and other prey organisms were greater in this season, thereby making it more suitable for the newborns.

Threats

Like any other aquatic or water body associated species, and the monitors of other habitats, there are several threats to the Common Water Monitors in the Dhaleswari River, which threaten the survival of the species.

Habitat loss and destruction. Most of the rivers of Assam (India) are under immense human pressure due to pollution, overfishing, extraction of water for agricultural and domestic uses, encroachment, and other uses; and the Dhaleswari River is no exception. The diversion of the river water to the Katakhal River by the sluice gate at Shahabad (Fig. 2D) has led to a decline in available water in the parent stream, and a loss of biodiversity. This water diversion also invites cultivation on the river banks. In several places on the banks, people made fences of bamboo or steel wire, thereby reducing the available space for the lizards on the banks. However, due to decreased river flow, erosion, and land-slides, many sections of the river could not be studied.

The Dhaleswari River is highly polluted due to dumping of domestic and agricultural wastes, sewage, carcasses of animals, and other refuse. All the wastes which are dumped continue to accumulate until the next monsoon. This leads to deterioration of water quality, and thereby causes depletion of aquatic diversity and prey species, although it does not appear to harm the lizards directly.

Prey species depletion. The Dhaleswari River does not maintain water flow in the winter months, and it fully dries up gradually upstream. In addition, people make dikes in the banks to catch any remaining fish left in the river, and these dikes are used for human movement as well. Unfortunately, the upstream section of the river has been encroached and converted into fisheries, and flow in certain areas has been diverted resulting in extremely narrow channels (Fig. 2E). In this situation, prey species, mainly fishes and those dependent on the fishes, barely remain in the river. The severe prey depletion is thus among the most important threats to the Common Water Monitor in this river.

Hunting. Unfortunately, there were several instances of hunting of the Common Water Monitor throughout its range in the river. It is poached for meat, and also for oil. The tribal community people of the region, mainly the *Manipuri*, *Khasi*, *Mizo*, *Naga*, Tea tribes, and others, hunt the monitor as a protein source. The oil extracted



Fig. 3. A Common Water Monitor (*Varanus salvator*) killed for venturing into a human habitation at Rongpur 6, Hailakandi. It was subsequently buried. Photo by R.A. Barbhuiya.

from the base of the tail of the lizard, called *Shandar tel* ('Oil of the Monitor lizard' in the local language), is used as a sexual lubricant by man. Although the Bengali community people, who are the majority in the present study area, do not consume the lizard themselves, they catch any individuals that venture into the settlements and sell them to the tribes. A moderate sized individual may cost up to 1,000–1,500 INR (~14–21 USD), while the oil is sold at the rate of 500 INR/L (~7 USD).

Human-monitor lizard conflicts. During the monsoon months, the preferred sites of nesting or refuge are submerged, and thus the lizards venture into human habitations for foraging, mainly in the afternoon hours. They often prey on domestic poultry or venture into artificial ponds and prey on fishes. Thus, the local people who do not consume the meat of the lizard consider them to be pests and often kill them. Such killing is an age-old practice. In addition, many people (mainly children) believe that the lizard may physically harm them, or even kill them, and that the bite of the lizard is poisonous. Thus, the local people often harm the Common Water Monitor whenever it occurs within their reach. Despite occasional attacks on domestic animals and venturing into ponds, the lizard was not reported by any of the respondents to actually attack man. In 2011, one individual was caught from village Kanchanpur, and killed (Saifur Rahman Laskar, Kanchanpur, pers. comm.). According

to the interviewees, there were about 25 (range 20–30) incidences of hunting Common Water Monitors in 2014 in the study area. In the month of August 2015, as many as six cases of such hunting were recorded from the river section between Bowerghat and Monacherra, a stretch of ~5 km. On 5 September 2015, one lizard ~1.8 m long (Fig. 3) was killed from Rongpur 6 village for attacking poultry, and subsequently buried. However, since the responses of only 30 interviewees were taken, the exact number of incidences is estimated to be much higher than is indicated here.

Lack of research and awareness. Unfortunately, the Common Water Monitors are the least studied of the large animals in the region. To the best of our knowledge, there are no reports on the Common Water Monitors from this region, save for the few records simply reporting its occurrence, and no mentions in the literature of its occurrence in Dhaleswari River. Moreover, no specific awareness campaign has yet been undertaken to educate the locals, and the lizard remains among the most ignored wildlife of the region.

Discussion

The freshwater bodies of the world, both lotic and lentic, are habitats with highly diverse wildlife assemblages as well as immense anthropogenic pressures, which threaten the survival of these species (Dudgeon 2000). Amidst a myriad of threats, the Common Water Monitor still survives in habitats that are highly disturbed. In the Dhaleswari River of Hailakandi (Assam, India), there is still a good population of the species, inhabiting the upstream stretch of the river which represents about one-half of the ~110 km total length of the river course. This varanid used to be very common throughout the region in the 1970s. However, today the species is encountered only in the 56 km stretch between Bowerghat and Shahabad (Fig. 1). During the survey, 32 individual Common Water Monitors were directly sighted in this river section, for a linear density of 0.57 individuals/km of the river course (Table 1). However, the linear density of the active nests was found to be 3.84/km, which suggests that the actual number of lizards inhabiting the river section is quite a bit higher than the number of individuals seen.

No present record of the species in the Katakhal River could be found. Since this river is much larger, with greater width, depth, flow rate, and flow discharge compared to the Dhaleswari, it further supports our assumption that the Common Water Monitor prefers smaller river habitats (Fig. 2B). In the Katakhal River, due to high flow velocity and discharge, the banks are frequently eroded in the monsoons, and thus monitor nests would be damaged. Since the species appears to use its nests repeatedly (for more than one year), larger rivers with sandy banks and prevalent erosion and landslides (Fig. 2C) would not serve as good habitats, and thus the

monitors avoid these rivers. This survey reveals that the nests are made on high grounds, which is consistent with earlier reports (Biswas and Kar 1980). The direction of the nests was found to be turned upwards in the present study area. Biswas and Kar (1980) also mentioned that the monitor seals the nest hole after laying eggs by scraping up soil, and we speculated that the half-sealed nests found in the present study area are probably similarly sealed and contain eggs that were laid.

During the present field work, some other fauna from the river section were recorded, which are prey of either the lizard or its competitors, or which prey on the eggs and hatchlings of the Common Water Monitor. Other lizards recorded from the present survey were Indian Garden Lizard (*Calotes versicolor*), Tokay Gecko (*Gekko gecko*), Bronze Grass Skink (*Eutropis macularia*), and Many-lined Grass Skink (*Eutropis multidasciata*); and the snakes included Checkered Keelback (*Xenochrophis piscator*), Red-necked Keelback (*Rhabdophis subminiatus*), Indian Rat Snake (*Ptyas mucosa*), Banded Krait (*Bungarus fasciatus*), Greater Black Krait (*Bungarus niger*), King Cobra (*Ophiophagus hannah*), Monocled Cobra (*Naja kaouthia*), Common Water Snake (*Enhydryis enhydryis*), and Indo-Chinese Rat Snake (*Ptyas korros*). The amphibians included Common Asian Toad (*Duttaphrynus melanostictus*), Indian Skipping Frog (*Euphlyctis cyanophlyctis*), Indian Bull Frog (*Hoplobatrachus tigerinus*), and Common Tree Frog (*Polypedates teraiensis*). The fishes recorded from the river were *Sperata aor*, *Sperata seenghala*, *Channa punctatus*, *Notopterus notopterus*, *Clarias batrachus*, *Heteropneustes fossilis*, *Pethia ticto*, and *Anabas testudineus*. The common fishes cultured by the local people in the ponds and fisheries in the adjoining areas (which are often prey of the Common Water Monitor) were *Labeo rohita*, *Clarias gariepinus*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Gibelion catla*, *Hypophthalmichthys molitrix*, and *Labeo calbasu*.

The water monitor of Dhaleswari River faces immense threats, which include anthropogenic pressures in terms of over-fishing, construction of dikes, conversion of the river into fisheries (Fig. 2E), cultivation of crops, and fencing of potential cultivable areas on the bank. Further, the species is frequently hunted as well as persecuted as a pest (Fig. 3). Thus, habitat destruction, hunting, and retaliatory killings are the major conservation issues in the present study area. These threats, in addition to others, have extirpated the other majestic aquatic mega-fauna of the river, including the Ganges River Dolphin *Platanista gangetica gangetica* (Mazumder et al. 2014). In spite of all these potential pressures, the Common Water Monitor could survive well in the river thanks to certain attributes, most notably its higher adaptability to human-modified habitats and wider food niche. In fact, the Common Water Monitor of Dhaleswari River is more common in town areas, where the dumping of municipal wastes provides better provisions for this lizard.

Moreover, the many poultry farms on the river banks deliberately dispose of dead birds near the nests of the lizards. In some areas where the monitor is deliberately or incidentally provisioned, it is often concentrated.

The Indian Wildlife (Protection) Act (1972) listed the species under Schedule I, thereby conferring maximum legal protection. Although the IUCN regards this species to be stable and considers it 'Least Concerned' (LC), the status of the population in southern Assam (India) is decreasing. Thus, the river section between Shahabad and Bowerghat requires special care, by limiting anthropogenic pressures. The Assam Fishery Rules (1953) should be enforced, as they restrict fishing of brood fishes in the spawning season, and prohibit fishing using specific methods in specific seasons, including dewatering, which will enhance fish stock. The construction of dikes, encroachment, and pollution of the river should be checked, and sewage should be treated before discharge. Hunting should be strictly dealt with and the relevant legislation should be strictly enforced. The people who consume the meat and use the oil of the lizards, should be motivated to stop doing so through education and outreach programs. The ecology, population status, distribution, and threats of the Common Water Monitor need to be properly evaluated and a specific long-term conservation action plan should be devised. Government and non-government organizations should come forward to work together for the conservation of this lizard. Above all, we as human beings and the dwellers of the Indo-Burma Biodiversity hotspot region should endeavor to save our pristine wildlife assemblage, or else the Common Water Monitor will be the next large reptile, after the Gharial and Marsh Crocodile, to become extirpated from southern Assam, India.

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Muhammed Khairujjaman Mazumder is currently working as Assistant Professor in the Department of Zoology, Dhemaji College, Assam, India. Muhammed obtained his Ph.D. from the Department of Life Science and Bioinformatics, Assam University, Silchar, India, in the subject of Neurobiology. He has a keen interest in the natural history of Northeast India, particularly Assam, and has authored several articles in journals of international repute and book chapters on the natural history of Assam, as well as Northeast India. Muhammed is particularly interested in mammals, and both aquatic and water-dependent species, their habitats, ecology, and conservation.



Amir Sohail Choudhury developed a keen interest in wildlife in childhood, being motivated by Dr. Anwaruddin Choudhury (one of the eminent naturalists of Northeast India). Amir completed post-graduate work in Ecology and Environmental Sciences with a specialization in Wildlife. His research interests include studying the population dynamics and habitat ecology of the birds and mammals of Assam, India. He has authored several articles and book chapters on the natural history of Northeast India, particularly Assam. Further, Amir has documented reptiles from the southern part of Assam, with several publications to his credit. He is a freelancer, and is very interested in wildlife photography.



Rofik Ahmed Barbhuiya is a young naturalist who has been involved in the study and conservation of wildlife, especially primates, elephants, and birds, since his school years. Rofik is a post-graduate in Ecology and Environmental Science at Assam University, Silchar, India, and is currently pursuing his Ph.D. from the same department on the behavioral ecology of the Capped Langur of Assam (India). He has conducted several awareness programs for the conservation of Hoolock Gibbon, Phayre's Langur, Capped Langur, and elephants, and has several articles published in international journals.



Himabrata Chakravarty is currently serving as Associate Professor and Head, Department of Zoology, Srikishan Sarda College, Hailakandi, Assam, India. Himabrata was awarded a Ph.D. by Assam University, Silchar, India, and his research interests include the ecology of birds, mammals, and reptiles. He has authored several articles and book chapters on the wildlife of Assam (India), and has been engaged in rescue, rehabilitation, and conservation programs for a long time. Himabrata also has a keen interest in wildlife photography.



Badruzzaman Barbhuiya is currently working as an Assistant Chemist in the District Level Laboratory, Public Health Engineering Department, Government of Assam, Hailakandi, Assam, India. Badruzzaman has a Chemistry background, and a keen interest in studying water quality and habitat ecology of aquatic species in southern Assam. He has experience in the analysis of water parameters in the region, and the mitigation of issues related to drinking water quality. With immense concern for the environment and climatic change, he remains involved in various awareness activities with NGOs.



Climatic niche, natural history, and conservation status of the Porthole Treefrog, *Charadrahyla taeniopus* (Günther, 1901) (Anura: Hylidae) in Mexico

¹Raciel Cruz-Elizalde, ²Itzel Magno-Benítez, ³Christian Berriozabal-Islas, ²Raúl Ortiz-Pulido, ^{2,*}Aurelio Ramírez-Bautista, and ⁴Raquel Hernández-Austria

¹Museo de Zoología “Alfonso L. Herrera”, Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM). A.P. 70-399, Ciudad de México CP 04510, MÉXICO ²Laboratorio de Ecología de Poblaciones, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Km 4.5 carretera Pachuca-Tulancingo, 42184, Mineral de La Reforma, Hidalgo, MÉXICO ³Programa Educativo de Ingeniería en Biotecnología. Universidad Politécnica de Quintana Roo. Av. Arco Bicentenario, M 11, Lote 1119-33, Sm 255, 77500 Cancún, Quintana Roo, MÉXICO ⁴Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-153, 04510 Ciudad de México, MÉXICO

Abstract.—Amphibian species of the family Hylidae exhibit a high degree of endemism in Mexico. To better understand ongoing declines of many amphibian populations, especially for endemic species that are particularly vulnerable to extinction, information on diverse aspects of their biological makeup is required, including their ecology. This study provides an analysis of the distribution, natural history, feeding habits, reproduction, morphology, and conservation status of *Charadrahyla taeniopus*, a species endemic to central Mexico. The distribution of this species extends along the Sierra Madre Oriental, primarily in cloud forest. Based on changes in climatic niche, decreases of 14.14% and 37% of its distributional range are predicted to occur by the years 2050 and 2070, respectively. An examination of the stomach contents from 31 adults and two juveniles revealed plant materials and arthropods as major parts of their diet. *Charadrahyla taeniopus* is sexually dimorphic in size. Females were larger than males, and after correcting for body size, females had larger jaws than males. Based on guidelines proposed by national legislation (NOM-059), we propose that this species should continue to be classified as Threatened. Further studies are necessary to classify it in a high conservation category by international legislation (IUCN) guidelines, due to the high vulnerability indicated by the Environmental Vulnerability Score, which is caused by an accelerated loss of habitat. *Charadrahyla taeniopus* is a good model for analyzing the conservation status of hylid frogs from temperate areas and in highly transformed environments, as this species exemplifies the conservation status of endemic amphibians in central Mexico.

Keywords. Amphibians, Central America, cloud forest, diet, morphology, reproduction

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Introduction

Scientists are concerned about the worldwide decline of amphibians associated with habitat degradation (Delia et al. 2013; Wilson et al. 2013), which is caused by a variety of factors, including shifts in land use, increased pollution, and the splitting and fragmentation of habitat (Becker et al. 2007; Ochoa-Ochoa et al. 2009; Cruz-Elizalde et al. 2015). As a result, many species are globally threatened (Lips et al. 2004; Ochoa-Ochoa et al. 2009) by human activities that are destroying habitat, leading to population decline, extirpation, or even species extinction (Delia et al. 2013). The creation of

regulations and laws at national and international levels, and resources such as the Red List of the International Union for the Conservation of Nature (IUCN), have been important tools for the conservation and management of biodiversity (Wilson et al. 2013).

In Mexico, flora and fauna are protected by the NOM-059, a regulation that provides a way to evaluate the threat level or conservation status of species through a nationally-recognized method: the *Método de Evaluación del Riesgo de Extinción de las Especies Silvestres en México* (method for evaluation of the extinction risk of wild species, MER; DOF 2010). MER has been used to evaluate the conservation status of diverse plant taxa,

Correspondence. *ramibautistaa@gmail.com



Fig. 1. Female individual of *Charadrahyla taeniopus* (A) in a cloud forest and (B) at Tenango de Doria, Hidalgo, Mexico. Photos by Uriel Hernández-Salinas (A) and Raciél Cruz-Elizalde (B).

but major groups of animals, including many species of amphibians, have not yet been evaluated (DOF 2010). Moreover, the lack of knowledge of the distribution and natural history of amphibian species populations impedes the proper application and evaluation of the MER. For example, *Lithobates johnei* (Blair, 1965) is an endemic species that is considered endangered, and was reported as extirpated at the type locality in Palictla, San Luis Potosí, Mexico (DOF 2010; Campos-Rodríguez et al. 2012). However, Hernández-Austria et al. (2015) found several robust populations of the species in the state of Hidalgo.

According to MER, conservation assessments should be updated to include current data and to focus on such critical factors as population density, reproductive period, and natural history (Wilson et al. 2013). One method for assessing conservation status that takes these factors into consideration is the Environmental Vulnerability Score (EVS). It is based on an algorithm proposed by Wilson et al. (2013) for amphibian species inhabiting Mexico, and has been accepted for biodiversity and conservation studies of this group at a variety of spatial scales (Johnson et al. 2015; Mata-Silva et al. 2015; Lemos-Espinal et al. 2018a,b). Use of this index has enabled better estimates of the conservation status of amphibian species at regional (Cruz-Elizalde et al. 2015, 2016) and state levels

(Johnson et al. 2015; Mata-Silva et al. 2015) by utilizing information on ecological distribution and reproductive modes (Wilson et al. 2013).

Approximately 99 species of the family Hylidae occur in Mexico (Wilson et al. 2013; Parra-Olea et al. 2014; Canseco-Márquez et al. 2017; Johnson et al. 2017; Jiménez-Arcos et al. 2019), and 68 of these are endemic to the country (Parra-Olea et al. 2014; Caviedes-Solis et al. 2015; Canseco-Márquez et al. 2017; Johnson et al. 2017). Many of the endemic species are distributed in temperate and tropical environments (Flores-Villela et al. 2010; Delia et al. 2013). The family Hylidae exhibits a remarkable species richness and diversity in montane regions (Duellman 2001; Flores-Villela et al. 2010), which are at high risk because of a shift in land use from forests to agroecosystems such as shade coffee plantations and grazing areas (Ochoa-Ochoa et al. 2009; Santos-Barrera and Urbina-Cardona 2011; Murrieta-Galindo et al. 2013). Furthermore, the temperate environments of Mexico are expected to be affected by climate change, leading to a decrease in species richness and diversity (Lips et al. 2004; Urbina-Cardona and Flores-Villela 2010).

The genus *Charadrahyla* (Faivovich et al. 2005) is composed of ten species (Frost 2019) which inhabit the highlands of the Sierra Madre Oriental, Sierra Madre Occidental, and the sierras of Oaxaca and Chiapas (Duellman 2001; Campbell et al. 2009; Frost 2019). Little is known about their ecology and natural history, so most of the species are assigned to categories of high extinction risk by the IUCN. Four of the species, *C. juanitae* (Synder, 1972), *C. nephila* (Mendelson and Campbell, 1999), *C. pinorum* (Taylor, 1937), and *C. taeniopus* (Günther, 1901) are in the Vulnerable category; *C. chaneque* (Duellman, 1961) is Endangered; *C. altipotens* (Duellman, 1968) and *C. trux* (Adler and Dennis, 1972) are Critically Endangered; and *C. sakbah* (Jiménez-Arcos, Calzada-Arciniega, Alfaro-Juantorena, Vázquez-Reyes, Blair, and Parra-Olea, 2019), *C. esperancensis* (Canseco-Márquez, Ramírez-González, and González-Bernal, 2017), and *C. tecuani* (Campbell, Blancas-Hernández, and Smith, 2009) have not been evaluated (IUCN 2019). According to NOM-059-SEMARNAT-2010, *C. altipotens*, *C. chaneque*, and *C. pinorum* are in the Subject to Special Protection (Pr) category; *C. juanitae*, *C. taeniopus*, and *C. trux* are Threatened (A); and the remaining four have not been evaluated (DOF 2010).

Charadrahyla taeniopus (Fig. 1A) inhabits undisturbed cloud forests (Fig. 1B) and pine-oak forests in the Sierra Madre Oriental (Duellman 2001; Kaplan and Heimes 2015), at elevations from 1,100 to 1,200 m in the states of Hidalgo, Puebla, and Veracruz (Duellman 2001). To date, knowledge about the potential distribution, ecology, reproduction, and natural history of this species is limited (Duellman 2001; Kaplan and Heimes 2015). The primary goal of this study is to assess changes in

the current and future (2050 and 2070) climatic niche of this species in Mexico. Secondary goals include characterizing the feeding habits, basic reproductive parameters, morphological variation, and conservation status of this species. This information can be used to develop future conservation strategies in environments with high species numbers and endemism, such as the cloud forests of the Sierra Madre Oriental (Ponce-Reyes et al. 2012).

Materials and Methods

Data Collection

Occurrence data for *C. taeniopus* were obtained from (i) the databases of the Global Biodiversity Information Facility (GBIF), the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and HerpNet; (ii) records from publications by Duellman (2001) and Campbell et al. (2009); and (iii) specimens from field work (sporadic collecting in the state of Hidalgo from 2008 to 2016) deposited in the Colección Herpetológica, Centro de Investigaciones Biológicas at the Universidad Autónoma del Estado de Hidalgo (Appendix 1).

Climatic Niche Modelling

The 37 unique occurrence records of *C. taeniopus* were used to generate climatic niche models. For this study, climate information was obtained from the 19 current climate layers available in the WorldClim database version 1.4 (Hijmans et al. 2005). These climate layers contain the averages of meteorological conditions recorded from North America, with a spatial resolution of 2.5 arc-min. To avoid overrepresentation of environmental variables, a bivariate correlation analysis was carried out with the aim of reducing multicollinearity among the input variables (Merow et al. 2013; Varela et al. 2014). For variables that were highly correlated ($r > 0.7$), the variable was chosen that exhibited the greatest variation or that represented the greatest biological meaning for the actual distribution of the species (e.g., temperature or precipitation). After this procedure, eight climate variables were retained: annual mean temperature (BIO 1), temperature seasonality (standard deviation $\times 100$, BIO 4), maximum temperature of the warmest month (BIO 5), minimum temperature of the coldest month (BIO 6), annual temperature range (BIO 5–BIO 6, BIO 7), mean temperature of the wettest quarter (BIO 8), mean temperature of the coldest quarter (BIO 11), and precipitation in the driest month (BIO 14; Hijmans et al. 2005). Subsequently, MAXENT was used, the selected climate variables were projected onto a map of Mexico and climate change scenarios were estimated (Phillips and Dudik 2008). Each model was replicated 100 times, the maximum number of repetitions allowed by available

computing power (Dambach and Rödder 2011). Average models for the present and for the future years 2050 and 2070 were then obtained. Current and future projections were estimated using the CCSM-GCM model under the greenhouse concentration scenario RCP8.5, which represents a pessimistic scenario ($RCP8.5 = +8.5 \text{ W/m}^2$).

Finally, to assess the impact of climate change on habitat suitability for the species, the percent change between current and future potential distribution areas was estimated. To determine habitat suitability, the formula $\% \text{ change} = [(S_1 - S_0)/S_0] \times 100\%$ was used, where S_0 is the total area that the species occupies in the country according to the current distribution scenario, and S_1 is the total area that the species could occupy in the country under future climate change conditions (Gutiérrez and Trejo 2014).

Diet

Stomach contents were removed from 31 adults and two juveniles (Appendix 1), and a stereomicroscope was used to classify all identifiable organisms to order, including plant material. The number of prey items in each stomach was tallied (n), the number of stomachs with each prey category (i) was determined (F_i), and the percentage of stomachs with prey category i (%F) was calculated. Also, the number of prey items belonging to each prey category (N) and the numerical percentage of total abundance (%N) represented by each prey category were determined. Then the volume of each prey item (mm^3) was calculated using the formula for an ellipsoid (Selby 1965; Duré and Kehr 2004; Duré et al. 2009): $V = 4/3 \pi (\text{length}/2) (\text{width}/2)^2$. The food importance index (I) was calculated using the formula of Biavati et al. (2004), which is $I = (F\% + N\% + V\%)/3$, where $F\%$ is the percentage of occurrence, $N\%$ is numerical percentage, and $V\%$ is volumetric percentage. The trophic niche amplitude was measured with Levin's standardized index using the formula $B_A = ((1/\sum p_i^2) - 1)/(n - 1)$, where p_i is the proportion of each prey category with respect to the total number of prey found in each sex, and n is the number of prey categories in the diet of individuals (Hurlbert 1978). The overlap in dietary composition between sexes was analyzed with Pianka's index (1986): $O_{jk} = \sum p_{ij} p_{ik} / \sum p_{ij}^2 \sum p_{ik}^2$, where p_{ij} and p_{ik} are the numerical proportions of prey belonging to the i^{th} category that was used by organisms (sexes) j and k (Pianka 1986; Gadsden and Palacios-Orona 1997). Analyses of amplitude and overlap for males, females, and juveniles were carried out in the Ecological Methodology v. 6.1.1. program (<http://www.exetersoftware.com> [Accessed: 26 January 2016]).

Reproduction

Thirty-one preserved adults (11 females and 20 males; Appendix 1) were examined to assess reproductive parameters. Fat bodies and livers from all individuals,

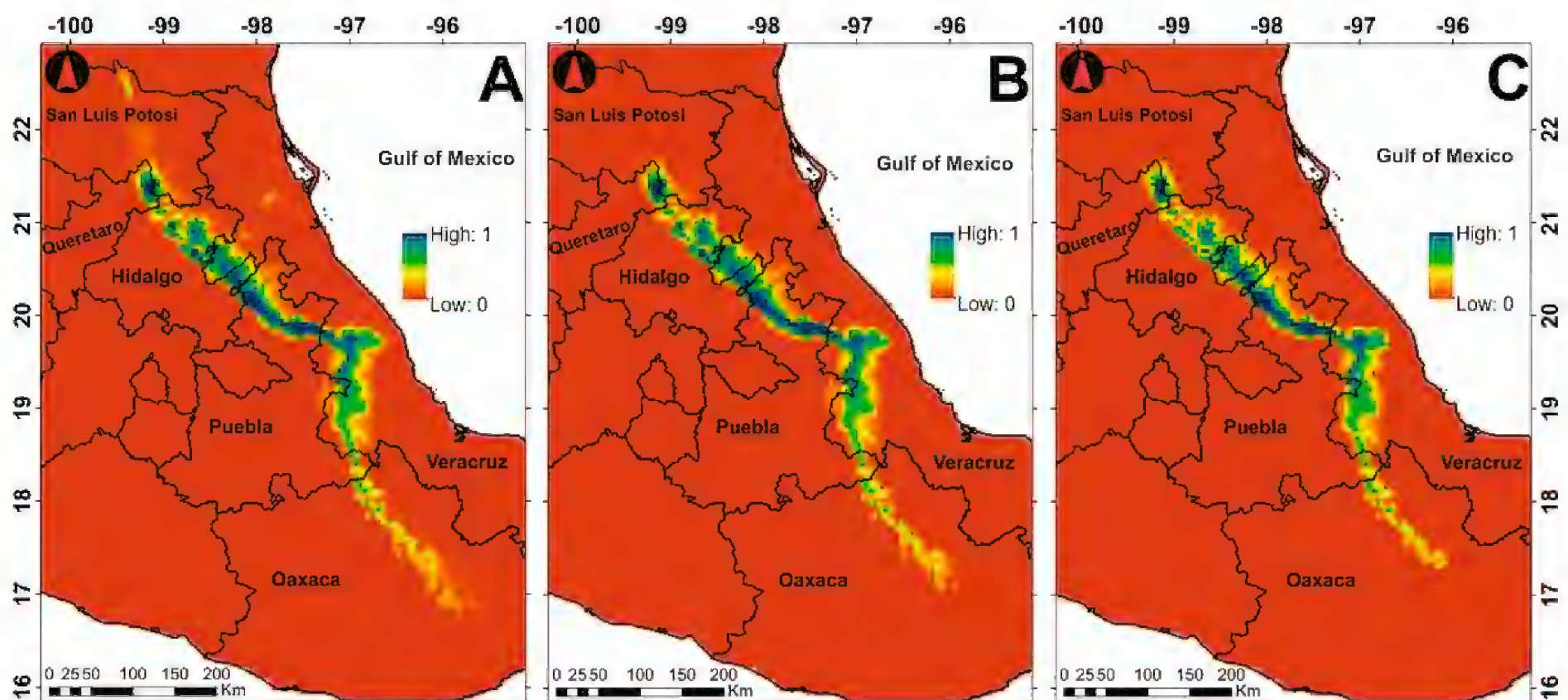


Fig. 2. Current (A), and future 2050 (B) and 2070 (C) scenarios of the climatic niche of *Charadrahyla taeniopus* in Sierra Madre Oriental province (degraded area). The color scale indicates the probability of occupancy for the species.

and egg mass in females or testes mass in males were weighed to the nearest 0.0001 g using an analytical balance. For males, the length and width of testes were measured to the nearest 0.01 mm with a digital caliper, and testicular volume was calculated using the formula for an ellipsoid (Selby 1965; Duré and Kehr 2004). In females, 10% of the total egg mass was excised from the oviducts, and the eggs contained therein were counted. The eggs were counted in a Petri dish with water using a stereomicroscope. Since the data were not normally distributed, Spearman's correlation test was used to evaluate correlations between body size and either egg mass or testicular mass. A Mann-Whitney U test was performed to evaluate the differences in mass, volume, length, and width of the testes.

Morphology

For all adult specimens (11 females and 20 males), digital calipers were used to measure the following (each ± 0.1 mm): snout-vent length (SVL), internarial distance (IND), eye diameter (ED), interorbital diameter (IOD), tympanum diameter (TD), head length (HL), head width (HW), head height (HH), jaw length (JL), jaw width (JW), length from forearm to the fourth finger (LFFT), forearm length (FOL), length of the humerus (LHU), thigh length (THL), tibia length (TL), and foot length (FL; Watters et al. 2016). The normality of the data was checked with a Kolmogorov-Smirnov test. Since the data were normal, an analysis of covariance (ANCOVA) was conducted to analyze sexual dimorphism. The function of the ANCOVA was to eliminate, through linear regressions, the effect of SVL (covariate) on the dependent variables (IND, ED, IOD, TD, HL, HW, HH, JL, JW, LFFT, FOL, LHU, THL, TL, and FL), and to check whether the regression slopes were different between the sexes (factor; Zar 2009). The data are presented as mean ± 1 SE.

Conservation Status

Conservation status was summarized by consulting Mexican regulations (NOM-059-SEMARNAT-2010; DOF 2010) and the IUCN (2019) Red List, and an Environmental Vulnerability Score (EVS; Wilson et al. 2013) was generated. The EVS recognizes three categories of risk: low (3–9 points), medium (10–13), and high (14–19). The score is the result of adding points assigned to features of a species based on three criteria: (i) extent of the geographic distribution, (ii) extent of ecological distribution (vegetation types used), and (iii) type of reproductive mode (Wilson et al. 2013).

Results

Areas of Occupation and Exchange Rates of Climate Availability

The distribution of *C. taeniopus* was restricted to cloud forests in the central region of the Sierra Madre Oriental (Fig. 2A–C) in the states of Hidalgo, Puebla, Veracruz, and the northern portion of Oaxaca, Mexico. The analysis of habitat occupancy under current conditions and in the future (to 2050 and 2070), showed a general loss of climatic niche in much of the range of *C. taeniopus*. This loss occurred in temperate areas, where the current area of occupation (18,262.23 km²) will decrease to 15,678.45 km² by 2050, which represents a habitat availability decrease of 14.14% (Fig. 2B); and to an area of 11,032.93 km² by the year 2070, which represents a habitat availability decrease of 37.08% (Fig. 2C).

Diet

Fourteen taxa were identified in the stomachs, with 11 taxa present in both males and females (Table 1). The

Table 1. Diet of *Charadrahyla taeniopus*, for the species and by sex. I = Food Importance Index, N = number of prey, %N = percentage number of prey, F = number of stomachs with prey category indicated, %F = percentage of stomachs with prey category indicated, V = volume of prey category, %V = percentage of volume of prey category.

Prey category	Total (n = 33)							Female (n = 13)							Male (n = 20)						
	N	%N	F	%F	V	%V	I	N	%N	F	%F	V	%V	I	N	%N	F	%F	V	%V	I
Arthropoda																					
Arachnida																					
Araneae	5	6.94	5	7.81	1821.6	6.33	7.03	1	3.85	1	3.85	301.59	3.26	3.65	4	8.70	4	10.53	1,520.01	7.78	9.00
Acari	4	5.56	4	6.25	80.11	0.28	4.03	3	11.54	3	11.54	79.59	0.86	7.98	1	2.17	1	2.63	0.52	<0.01	1.60
Solifugae	1	1.39	1	1.56	8,616.36	29.93	10.96								1	2.17	1	2.63	8,616.36	44.12	16.31
Insecta																					
Coleoptera	5	6.94	4	6.25	631.99	2.20	5.13	1	3.85	1	3.85	6.28	0.07	2.59	4	8.70	3	7.89	625.70	3.20	6.60
Coleoptera larva	1	1.39	1	1.56	980.18	3.40	2.12	1	3.85	1	3.85	980.18	10.59	6.09							
Dermaptera	3	4.17	3	4.69	28.27	0.10	2.98	2	7.69	2	7.69	27.23	0.29	5.23	1	2.17	1	2.63	1.05	0.01	1.60
Diptera	1	1.39	1	1.56	0.52	<0.01	0.98	1	3.85	1	3.85	0.52	0.01	2.57							
Hemiptera	3	4.17	3	4.69	28.27	0.10	2.98	1	3.85	1	3.85	18.85	0.20	2.63	2	4.35	2	5.26	9.42	0.05	3.22
Hymenoptera	2	2.78	2	3.13	97.39	0.34	2.08	2	7.69	2	7.69	97.39	1.05	5.48							
Formicidae	10	13.89	6	9.38	114.14	0.40	7.89	1	3.85	1	3.85	0.52	0.01	2.57	9	19.57	5	13.16	113.62	0.58	11.10
Lepidoptera larva	3	4.17	1	1.56	502.66	1.75	2.49								3	6.52	1	2.63	502.66	2.57	3.91
Orthoptera	20	27.78	19	29.69	9,655.18	33.54	30.33	8	30.77	8	30.77	4,780.47	51.64	37.73	12	26.09	11	28.95	4,874.72	24.96	26.66
Diplopoda	1	1.39	1	1.56	466.53	1.62	1.52								1	2.17	1	2.63	466.53	2.39	2.40
Plant material (leaves)	13	18.06	13	20.31	5,764.31	20.02	19.46	5	19.23	5	19.23	2,964.10	32.02	23.49	8	17.39	8	21.05	2,800.21	14.34	17.59
Totals	72	100	64	100	28,787.51	100		26	100	26	100	9,256.72	100		46	100	38	100	19,530.80	100	

Table 2. Mean values $\pm$ 1 SE, and range in parentheses, of morphological characteristics of adult *Charadrahyla taeniopus* (SVL = snout-vent length, IND = internarial distance, ED = eye diameter, IOD = interorbital diameter, TD = tympanum diameter, HL = head length, HW = head width, HH = head height, JL = jaw length, JW = jaw width, LFFT = length from forearm to the fourth finger, FOL = forearm length, LHU = length of the humerus, THL = thigh length, TL = tibia length, FL = foot length). Comparisons were made with ANCOVA, with SVL as the covariate.

Characteristics	Female ($n = 11$)	Male ($n = 20$)	ANCOVA		
			<i>F</i>	df	<i>P</i>
IND	5.58 $\pm$ 0.24 (3.87–7.08)	4.94 $\pm$ 0.18 (2.91–6.72)	1.133	1, 28	0.296
ED	6.22 $\pm$ 0.23 (5.06–7.91)	5.86 $\pm$ 0.21 (4.86–8.80)	0.065	1, 28	0.800
IOD	7.02 $\pm$ 0.36 (4.24–8.74)	5.21 $\pm$ 0.39 (1.57–8.18)	5.396	1, 28	0.027
TD	3.75 $\pm$ 0.17 (2.64–4.62)	3.29 $\pm$ 0.26 (1.99–6.85)	0.457	1, 28	0.504
HL	19.82 $\pm$ 0.97 (12.60–24.09)	18.46 $\pm$ 0.38 (15.81–22.91)	0.002	1, 28	0.966
HW	19.99 $\pm$ 0.70 (13.94–22.51)	16.45 $\pm$ 0.32 (14.01–19.41)	24.521	1, 28	<0.001
HH	8.14 $\pm$ 0.36 (5.56–9.19)	6.83 $\pm$ 0.32 (4.95–9.08)	3.553	1, 28	0.069
JL	17.28 $\pm$ 0.66 (12.33–20.68)	15.33 $\pm$ 0.26 (13.57–17.45)	6.433	1, 28	0.017
JW	19.91 $\pm$ 0.80 (13.05–22.99)	17.22 $\pm$ 0.31 (15.20–20.87)	19.166	1, 28	<0.001
LFFT	16.83 $\pm$ 1.00 (12.03–21.05)	15.60 $\pm$ 0.53 (11.58–20.37)	0.003	1, 28	0.959
FOL	13.78 $\pm$ 0.65 (9.44–16.91)	13.12 $\pm$ 0.29 (11.08–15.58)	1.387	1, 28	0.249
LHU	16.38 $\pm$ 0.84 (10.77–20.80)	16.07 $\pm$ 0.51 (10.48–19.26)	2.365	1, 28	0.135
THL	31.32 $\pm$ 1.44 (20.21–38.05)	30.17 $\pm$ 0.65 (25.19–37.96)	0.927	1, 28	0.344
TL	32.68 $\pm$ 1.24 (22.02–37.08)	30.68 $\pm$ 0.54 (27.84–37.26)	0.016	1, 28	0.900
FL	43.06 $\pm$ 1.77 (30.21–51.14)	37.92 $\pm$ 2.17 (34.38–49.26)	0.035	1, 27	0.853

most important prey categories, according to the values of food importance for the species and for each sex, were orthopterans, plant material (leaves), and ants (Table 1). The overlap in diet between the sexes was high ($O_{jk} = 0.822$; 63.21–100%), with males presenting a slightly higher value ($B = 0.526$) of diet niche breadth than females ($B = 0.504$).

Reproduction

The mean number of eggs was 722 ± 277.53 (range 426–1,138, $n = 11$). There was no correlation between female SVL and either number of eggs ($r_s = 0.09$, $P = 0.79$, $n = 11$) or egg mass in females ($r_s = 0.09$, $P = 0.79$, $n = 11$). There were no differences among weights, lengths, widths, or volumes of the testes ($P > 0.05$ in all cases). The average weight, length, width, and volume of the right testis was 0.198 g, 14.78 mm, 6.05 mm, and 305.57 mm³, respectively; and for the left testis the averages were 0.204 g, 14.63 mm, 6.0 mm, and 293.25 mm³, respectively. There was no correlation between SVL and testicular volume ($r_s = 0.31$, $P = 0.17$, $n = 20$), but there was a positive correlation between SVL and testicular mass in males ($r_s = 0.49$, $P = 0.02$, $n = 20$).

Morphology

Five of the 15 characteristics measured exhibited sexual dimorphism, with females higher than males in SVL,

IOD, HW, JL, and JW (Table 2). Females (mean SVL = 63.94 ± 2.35 mm; range 45.27–74.31, $n = 11$) were larger than males (mean SVL = 59.70 ± 1.09 mm, range 52.90–71.05, $n = 20$; $U = 60$, $P = 0.04$).

Conservation Status

Charadrahyla taeniopus is listed in conservation standards (DOF 2010; IUCN 2019) as being in high risk categories. According to the Mexican Standard NOM-059-SEMARNAT-2010 (DOF 2010), the species is considered to be Threatened. The IUCN Red List of Threatened Species places the species in the Vulnerable category, with a distributional area less than 20,000 km² in fragmented environments and with declining populations (status B1ab[iii]; IUCN 2019). In the EVS, it was classified as a medium environmental vulnerability species, with a value of 13 points. This EVS category was calculated from: (i) its distribution in Mexico, but not exclusive to the type locality (5 points), (ii) its occurrence in two vegetation types (pine-oak and cloud forest, 7 points), and (iii) a reproductive mode with egg laying in lentic or lotic water bodies (1 point; Wilson et al. 2013).

Discussion

Conserving native populations of tree frogs at a local scale requires information on their ecological distribution,

feeding habits, reproduction, and morphology (Delia et al. 2013; Toledo et al. 2014). The analyses reported here suggest that the distribution of *C. taeniopus* will potentially decrease during the next 50 years. Microhabitats in currently occupied habitats (montane environments) are subject to change because of temperature and moisture shifts, and also because of changes in vegetation cover associated with high deforestation rates (Kaplan and Heimes 2015) and potential climate change, including shifts in temperature and moisture (Ponce-Reyes et al. 2012). For example, several authors including Pineda and Halffter (2004), Pineda et al. (2005), and Murrieta-Galindo et al. (2013), have suggested that the existence of abundant vegetation and native shrub cover provide appropriate humidity and temperature conditions for the permanence of hyliid frogs in temperate environments such as cloud forests. If the abiotic and biotic conditions change in the forests inhabited by *C. taeniopus*, this species could be negatively affected. Loss of climatic niche in our models is consistent with that reported by Roxburgh et al. (2004). These authors mentioned that the expected changes could generate ecological scenarios that will delimit the overall distribution of arboreal species from cloud forests (Roxburgh et al. 2004; Pineda et al. 2005), and therefore could affect their associations with their environment (Urbina-Cardona and Flores-Villela 2010; Ponce-Reyes et al. 2012).

In addition to the above considerations, the thermal tolerances of anurans in high elevation or low temperature environments can determine the presence and distribution of their populations (Wells 2007). The hyliid frogs are an example of this, as their limits of distribution are in high latitude regions such as the arid and semi-arid climates of northern Mexico (Wiens et al. 2006). This may be the result of the thermal tolerances that hyliid species show in temperate environments, which are different from those of species that occur in tropical environments (Navas 2006; Wells 2007). To date, there are no studies of thermal tolerances or maximum/minimum temperature limits for *C. taeniopus*; therefore, it is very difficult to know the behavior of individuals and/or populations of this species in their distribution area. Future field studies, and *in situ* and laboratory experiments on thermal preferences are therefore necessary for this species. They could complement the results obtained in the potential distribution model of the species, enabling the analysis of variables that could be interacting to a greater degree with the biology of the organism, and improving determinations of the distribution range of the species (Gross and Price 2000; Wiens et al. 2006).

Ochoa-Ochoa et al. (2009), stated that in addition to the loss of vegetation, climate change is a determining factor in the loss of amphibian species in conserved environments, mainly in sites outside of natural protected areas (NPAs). The Sierra Madre Oriental Corridor occupies large areas of cloud forest, a type of environment that is highly threatened by the effects of

climate change (Ponce-Reyes et al. 2012), and in which the known distribution of the species is not included in any NPA (IUCN 2019). This shows the importance of evaluating the distribution of highly vulnerable hyliid frogs throughout the potential distribution range based on climatic niche models and climate change scenarios. The results are worrisome, because despite the fact that amphibian richness in Mexico is high (Johnson et al. 2015, 2017), more than 50% of the species are listed in high vulnerability categories by the IUCN (Delia et al. 2013; Caviedes-Solis et al. 2015; IUCN 2019; Johnson et al. 2017). For example, recent studies have found that some mountain hyliid species have not been recorded over prolonged periods of time (Delia et al. 2013; Caviedes-Solis et al. 2015). Due to multiple factors, such as vegetation loss, pollution, and in particular climate change, populations of these species tend to occur in highly vulnerable sites (Lips et al. 2004; Stuart et al. 2004). Therefore, the species that inhabit this type of environment (cloud forest, pine-oak) are highly threatened (Ochoa-Ochoa et al. 2009; Caviedes-Solis et al. 2015).

In addition to habitat fragmentation and climate change, the presence of the pathogenic fungus *Batrachochytrium dendrobatidis* Longcore, Pessier, and Nichols, 1999 (*Bd*) has contributed to amphibian population and species losses in Mexico (Mendoza-Almeralla et al. 2015, 2016) and other regions of the world (Lips et al. 2003; Fisher et al. 2009). However, *Bd* has not been detected thus far in *C. taeniopus* (Murrieta-Galindo et al. 2014; Hernández-Austria 2017). Therefore, further studies are needed to examine the potential presence of *Bd* in *C. taeniopus* populations through their distribution area (Hernández-Austria 2017).

The lack of information on the natural history of this species inhibits the development of strategies for its conservation (Toledo et al. 2014). The data presented here on diet provide valuable information on the basic ecology of *C. taeniopus*. The diet of this species consists of orthopterans, plant material, and ants, and there is a high degree of overlap in diet between the sexes. In *C. taeniopus*, plant material is the second most important food item. This is particularly notable since the diet of most anuran species in Mexico consists primarily of arthropods (Ramírez-Bautista and Lemos-Espinal 2004; Suazo-Ortuño et al. 2007), and the ingestion of plant material, such as leaves and flowers, is usually considered to be accidental (Evans and Lampo 1996). In the case of *C. taeniopus*, further studies are necessary to determine if consumption of plant material (leaves) is accidental or part of their diet, which would be unusual, but not unprecedented. For example, some species of tree frogs, such as *Ptychohyla zophodes* Campbell and Duellman, 2000 (Luría-Manzano 2012) and *Xenohyla truncata* (Izecksohn, 1959) do consume large quantities of plant material, and the latter (*X. truncata*) has been reported as entirely omnivorous, consuming fruits, seeds,

and flowers (da Silva and Britto-Pereira 2006).

Egg number and the relative sizes of eggs vary greatly in amphibians (Vitt and Caldwell 2009), and they are often related to female body size (Jorgensen 1992; Hartmann et al. 2010). The data presented here show that egg number is not related to female body size in *C. taeniopus*. This may be due to the fact that its reproductive period may have a longer duration, and the sample size obtained from the collections only reflects the behavior of the females in the first part of the year (March–April), not in the entire reproductive period. Females with eggs were found throughout the year, and aggregations of individuals of both sexes and amplexus were observed in the field in August. This seasonal variation in the correlation between egg size and size of females has been reported for other anuran species such as *Leptodactylus fuscus* (Schneider, 1799), *L. podicipinus* (Cope, 1862), and *Dendropsophus nanus* (Boulenger, 1889) [Prado and Haddad 2005]. Furthermore, testicular mass, but not testicular volume, increases with larger SVL. These data suggest that larger males invest more energy in sperm production to have greater reproductive success (Byrne et al. 2002).

Most species of frogs (nearly 90%) are sexually dimorphic, with females being larger than males (Wells 2007), and *C. taeniopus* is no exception. The larger size of females compared to males is presumably associated with the potential to produce more eggs. However, no correlation was found between SVL of females and egg number. Another explanation for sexual size dimorphism could be differences in growth rates (Kupfer 2007), in which the growth rate of males is faster than that of females in order to reach sexual maturity at a smaller size and compete with other males for access to calling sites, thereby maximizing the number of matings (Kupfer 2007; Wells 2007). Also, considering the ecological hypothesis to explain the sexual dimorphism, the larger jaw size in females compared to the males might indicate a larger gape in females, which could allow for partitioning of food resources in terms of prey size (Luría-Manzano 2012). However, additional studies on microhabitat use, behavior, and reproduction are required before the ecological significance of the sexual dimorphism in *C. taeniopus* can be determined.

Based on the information about climatic niche, feeding habits, reproduction, and morphology, *C. taeniopus* is highly threatened because it is distributed in environments (i.e., cloud, oak, and pine-oak forests) that are currently being dismantled by fragmentation and climate change (Ponce-Reyes et al. 2012). As with other hylid frogs (Caviedes-Solis et al. 2015), *C. taeniopus* could face a rapid rate of population decline, as has occurred in other species inhabiting the temperate areas of cloud forest in Oaxaca (Delia et al. 2013; Mata-Silva et al. 2015), Chiapas (Johnson et al. 2015), and areas of the Sierra Madre Oriental (Flores-Villela et al. 2010). To add to the information presented in this study, additional

studies on demography, ecology, physiological tolerances to temperature, length of the reproductive period, effect of fragmentation on populations, and population dynamics of this species should be conducted in order to devise efficient conservation strategies for *C. taeniopus*, and other species of anurans that inhabit the temperate and tropical montane environments of central and southern Mexico (Delia et al. 2013; Caviedes-Solis et al. 2015).

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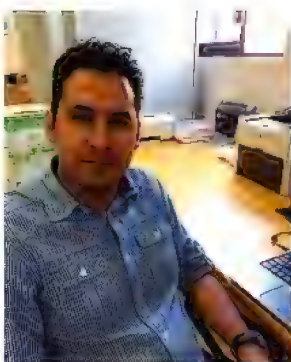
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Appendix 1. Voucher numbers of *Charadrahyla taeniopus* specimens analyzed in this study from Colección Herpetológica del Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Hidalgo, México. Year **2008**, September: CIB 5422–5425 (males), December: CIB 5426 (female); **2009**, April: CIB 5427 (female), June: CIB 5428 (male), CIB 5429–5430 (females), July: CIB 5430 (female), August CIB 5431 (male), CIB 5432 (female), October: CIB 5433–5435 (males), CIB 5436–5437 (juveniles, females), November CIB 5438 (female), CIB 5439 (juvenile, female); **2010**, June: CIB 5440 (male); **2011**, April CIB 5441 (female), CIB 5442–5443 (males), CIB 5444 (female), CIB 5445 (juvenile, female), CIB 5446 (male), May CIB 5447–5449 (males), June CIB 5450 (male); **2012**, March CIB 5451–5452 (females), CIB 5453 (male); **2015**, April CIB 5454–5455 (males), CIB 5456 (female).



Raciél Cruz-Elizalde is a Mexican herpetologist who received his B.Sc. in Biology, M.Sc. in Biodiversity and Conservation, and Ph.D. in Biodiversity and Conservation from the Autonomous University of Hidalgo State, Mexico. He is currently conducting a postdoctoral stay at the Department of Evolutionary Biology, Faculty of Science, National Autonomous University of Mexico (UNAM). Raciél is interested in the ecology, life history evolution, diversity, and conservation of amphibians and reptiles in Mexico. His current research includes life history evolution of diverse lizard species of the genus *Sceloporus*, conservation issues in natural protected areas, and analysis of ecological and morphological traits in the composition of amphibian and reptile species assemblages.



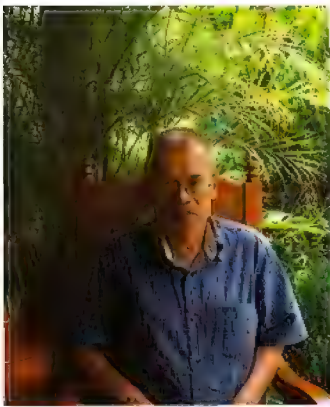
Itzel Magno-Benítez is a Mexican herpetologist. Itzel received her B.Sc. in Biology from the Autonomous University of Hidalgo State, Mexico, and she is interested in the ecology and conservation of amphibians and reptiles in Mexico. Her current research interests include the anthropic effects on conservation and ecology (feeding habits, reproduction, and morphological variation) of amphibians and reptiles in the temperate and arid environments of Mexico.



Christian Berriozabal Islas received a Ph.D. from the Autonomous University of Hidalgo State, Mexico. His research focuses on the diversity of amphibians and reptiles, conservation, and the effects of climate change on tropical lizards and kinosternid turtles.



Raúl Ortiz-Pulido is a Mexican scientist, amateur astronomer, scientific communicator, and artist (landscape photographer). Raúl received a B.Sc. degree from the Universidad Veracruzana (1994) and a doctorate in sciences from the Instituto de Ecología, A.C. (2000). He has been working with animal ecology since 1991, particularly the relationship between food abundance and individuals of different species. In 1997, Raúl started the Huitzil Mexican Journal of Ornithology and in 2002 the astronomical society of his current university. In 2005, he was elected president of CIPAMEX, a Mexican association of scientists. Raúl has written more than 200 scientific papers and given more than 250 talks, many of them popularizing science. He is a reviewer for 27 indexed scientific journals and associate editor of four of them. Currently, Raúl is associated with the Population Ecology Laboratory of the Universidad Autónoma del Estado de Hidalgo, where he has worked since 2001.



Aurelio Ramírez-Bautista began his herpetological career conducting research as an undergraduate student at the Los Tuxtlas Biological Field Station, Veracruz, Mexico. Aurelio received his B.Sc. in Biology from Universidad Veracruzana in Veracruz, Mexico, his M.Sc. and Doctorate degrees from the Universidad Nacional Autónoma de México (UNAM), and a postdoctoral appointment at the University of Oklahoma, Norman, Oklahoma, USA. Aurelio's main research involves studies on ecology, demography, reproduction, conservation, and life history evolution, using amphibians and reptiles of Mexico as models. He was president of the Sociedad Herpetologica Mexicana and is currently associate editor of *Mesoamerican Herpetology*. Aurelio is a professor at Universidad Autónoma del Estado de Hidalgo (UAEH), teaching population ecology, herpetology, and natural history of amphibians and reptiles.



Raquel Hernández-Austria is a biologist with an M.Sc. in Biodiversity and Conservation Sciences from the Universidad Autónoma del Estado de Hidalgo, Mexico. Raquel's interest is the study of amphibian and reptile diversity and conservation. For her undergraduate degree, she compared the food habits of two syntopic species of *Lithobates*, and for her Master's degree she evaluated the presence of *Batrachochytrium dendrobatidis* in anuran species of Hidalgo State. Raquel has participated in various Mexican congresses and is an author and co-author of many scientific papers related to amphibians and reptiles.



Detection of *Ophidiomyces ophiodiicola* at two mid-Atlantic natural areas in Anne Arundel County, Maryland and Fairfax County, Virginia, USA

^{1,*}Lauren D. Fuchs, ²Todd A. Tupper, ³Robert Aguilar, ¹Eva B. Lorentz, ²Christine A. Bozarth, ²David J. Fernandez, and ⁴David M. Lawlor

¹George Mason University, Department of Systems Biology, 10900 University Blvd, Manassas, Virginia 20110 USA ²Northern Virginia Community College, Division of Math, Science, Technologies and Business, 5000 Dawes Avenue Alexandria, Virginia 2231 USA ³Smithsonian Environmental Research Center, Fish and Invertebrate Ecology Lab, 647 Contees Wharf Road, Edgewater, Maryland 21037 USA ⁴Huntley Meadows Park, Natural Resource Management Division, 3701 Lockheed Blvd Alexandria, Virginia 22306 USA

Abstract.—Since the early 2000s, ophidiomycosis has been reported with increasing frequency and associated with widespread morbidity in numerous North American snake species. *Ophidiomyces ophiodiicola* (*Oo*), the etiologic agent of ophidiomycosis, has been detected in over 30 species throughout most of the eastern United States, as well as in Europe and Australia; however, it is suspected that the distribution of this pathogen may be underestimated due to a lack of standardized inventories. To contribute to the existing but limited data on ophidiomycosis in the mid-Atlantic United States, snakes were sampled for *Oo* at two natural areas in this region—one in Anne Arundel County, Maryland and one in Fairfax County, Virginia. *Ophidiomyces ophiodiicola* was detected at both study sites. Thirty-four of 61 (55.7%) samples across eight species tested positive for the pathogen, with the highest detection rates occurring in *Nerodia sipedon* (73.1%) and *Pantherophis alleghaniensis* (70%). *Ophidiomyces ophiodiicola* was detected in snakes with (71.4%) and without (34.6%) clinical signs of ophidiomycosis. These results support the need for both increased *Oo* monitoring throughout the region, and implementation of more standardized and unbiased sampling protocols.

Keywords. Colubridae, ophidiomycosis, population decline, Reptilia, Serpentes, snake fungal disease

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Introduction

Ophidiomycosis has emerged as a growing threat to snakes throughout much of North America (Dolinski et al. 2014; Allender et al. 2015; Lorch et al. 2016; Paré and Sigler 2016) and has been associated with widespread morbidity in numerous species (Guthrie et al. 2016; Lorch et al. 2016; Stengle 2018). The disease is attributed to *Ophidiomyces ophiodiicola* (*Oo*), a mycotic pathogen that is only known to infect snakes (Allender et al. 2015; Lorch et al. 2016; Paré and Sigler 2016). Clinical manifestations of infection (see Fig. 1) typically include scabs, crusty scales, superficial pustules, subcutaneous nodules, and dysecdysis (Dolinski et al. 2014; McBride et al. 2015; Tetzlaff et al. 2015). Ophidiomycosis infections are generally chronic, but mild; however, severe infections with high mortality have been reported in several viperid species (Allender et al. 2013; Sigler et

al. 2013; Sleeman 2013; Lorch et al. 2015, 2016; Stengle et al. 2018). The precise mechanisms that influence lethal outcomes of the disease are still unclear, but are likely multifaceted (Lorch et al. 2015; Guthrie et al. 2016).

Since 2006, ophidiomycosis has been increasingly documented, with cases of infection reported in at least 20 states, including Maryland and Virginia (Allender et al. 2015; Guthrie et al. 2016; Tupper et al. 2015, 2018, 2019). Despite growing reports of ophidiomycosis throughout the mid-Atlantic, systematic studies designed to assess its prevalence using non-incidental sampling methods are limited. The rising incidence of ophidiomycosis, coupled with habitat loss, pollution, and other anthropogenic stressors, poses an added challenge for snake conservation, underscoring the significance of ongoing disease monitoring (Franklinos et al. 2017; Kucherenko et al. 2018). The objective of this study was to assess the presence and prevalence of *Oo* at two mid-

Correspondence. *lfuchs@masonlive.gmu.edu



Fig. 1. The ventral scales of a symptomatic Northern Black Racer (*Coluber constrictor*) infected with *Ophidiomyces ophiodiicola*. This 41 g male was captured and swabbed on 20 May 2018 at Huntley Meadows Park. Its total length was 123.2 cm and snout-to-vent length was 94.6 cm. Photo by Eva Lorentz.

Atlantic natural areas located in Maryland and Virginia, USA. The results obtained contribute toward an improved understanding of the distribution and prevalence of ophidiomycosis in the region.

Materials and Methods

Area-constrained visual encounter searches (Crump and Scott 1994) were used to sample for *Oo* in snakes from Huntley Meadows Park (HMP; 38°45'36.57" N, 77°05'44.13" W; Fig. 2) in Fairfax County, Virginia, and at the Smithsonian Environmental Research Center (SERC; 38°53'17.41"N, 76°33'15.52" W; Fig. 3) in Anne Arundel County, Maryland, between 22 April 2018 and 9 October 2018. Snakes were hand-captured (wearing sterile nitrile gloves) and visually inspected for clinical signs of ophidiomycosis (Allender et al. 2011; Clark et al. 2011). Then, using a modified protocol developed by Allender et al. (2016), snake skins were sampled with sterile dry swabs (no. MW113, Medical Wire and Equipment Company, Durham, North Carolina, USA) from all craniofacial scales and along the entire ventral length of the body separately, swabbing each region five times, taking care to swab any lesions, pustules, nodules, or displaced scales on snakes which showed signs of infection (Allender et al. 2011, 2016). Swabs were stored in sterile 1.5 mL microcentrifuge tubes and kept frozen until molecular analysis. Prior to release, each snake was measured, weighed, and photographed to help in differentiating conspecifics. Aseptic techniques were employed and appropriate biosecurity protocols were followed (see Rzedkowska et al. 2016; VHS 2016) to limit the transmission of *Oo*.

For the *Oo* assay, DNA was eluted from the swabs using the Purification of Total DNA from Animal Tissues Protocol (Qiagen®, Valencia, California, USA). To ensure samples were not contaminated during the



Fig. 2. Location of Huntley Meadows Park (HMP) snake capture locations. Black markers = all samples positive; white markers = all samples negative; gray markers = samples either positive or negative.

extraction process, a negative control was used, which included all elements of the extraction mixture other than DNA. Following methods described by Allender et al. (2015), 2.5 µL of eluted DNA was combined with 12.5 µL Sso Advanced™ universal probes supermix (Bio-Rad, Hercules, California, USA), 1.25 µL of a combined target-specific primer (OphioITS-F and OphioITS-R)-probe, and water, creating a 25 µL reaction mixture. The DNA was amplified via qPCR using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA), with the following cycling parameters: 1 cycle at 50 °C for 2 min, 1 cycle at 95 °C for 10 min, 40 cycles of 95 °C for 15 sec and 60 °C for 60 sec, followed by a final cycle at 72 °C for 10 min.

For each round of qPCR, a positive control was included by adding 2.5 µL of a plasmid containing *Oo* (obtained from the Wildlife Epidemiology Laboratory at Illinois University at Urbana-Champaign, Illinois, USA) to a designated well containing the 22.5 µL mixture of primer-probe, and water (as described above). A well was also included for the negative control, which contained only the 22.5 µL mixture, but no DNA. These controls were used to determine whether the reaction mixture was prepared accurately, and to ensure that samples were not contaminated during qPCR preparation. Up to five rounds of qPCR were performed for each sample. A sample was considered positive if at least three rounds (per sample) had a lower cycle threshold (C_t) than the



Fig. 3. Location of Smithsonian Environmental Research Center (SERC) snake capture locations. Black markers = all samples positive; white markers = all samples negative; gray markers = samples either positive or negative.

lowest detected standard dilution for the positive control (Allender et al. 2016).

Sampling sites within the study areas (indicating locations of positive and negative samples) were plotted with ESRI ArcMap (version 10.6). Snake nomenclature corresponds with Crother et al. (2017). Tables and descriptive statistics were completed with Microsoft Excel for Office 365 (Microsoft Corporation, Redmond, Washington, USA).

Results

Sixty snakes (35 from HMP and 25 from SERC) across nine species were captured and swabbed (Table 1). Northern Watersnake (*Nerodia sipedon*) comprised the largest proportion ($n = 26$; 43.3%) of the captures. Eastern Ratsnake (*Pantherophis alleghaniensis*; $n = 10$), Common Ribbonsnake (*Thamnophis sauritus*; $n = 9$), and Eastern Wormsnake (*Carphophis amoenus*; $n = 7$) were also well-represented, comprising 16.7%, 15%, and 11% of the total snake sample, respectively. Northern Black Racer (*Coluber constrictor*; $n = 2$), Eastern Kingsnake (*Lampropeltis getula*; $n = 1$), Northern Ring-necked Snake (*Diadophis punctatus*; $n = 1$), Eastern Gartersnake (*Thamnophis sirtalis*; $n = 2$), and Dekay's Brownsnake (*Storeria dekayi*; $n = 2$) were all sparsely represented. *Ophidiomyces ophiodiicola* was detected in 33 snakes and in a shed skin of a Northern Black Racer, yielding an overall detection rate of 55.7%. More than half of the positive samples (55.9%) were from a single species—Northern Watersnake. Of the nine species sampled, Northern Watersnake had the highest detection rate (73.1%), followed closely by Eastern Ratsnake (70%). Northern Black Racer, Eastern Wormsnake, and Common Ribbonsnake were positive in 66.7%, 28.6%, and 11.1% of samples, respectively. Only one Eastern Kingsnake and one Northern Ring-necked snake were sampled, and both were positive. Dekay's Brownsnake was positive in one of two samples and Eastern Gartersnake was the only species that did not test positive for *Oo*. Twenty-five of the 35 (71.4%) snakes showing clinical signs tested positive for *Oo*, and nine of the 26 (34.6%) without clinical signs were *Oo* positive (Table 1). Prevalence varied between study locations, with 34.6% of snakes testing positive at HMP and 84.6% at SERC. Of the 34 snakes testing positive, *Oo* was detected in both swabs in 18 snakes (52.9%) and in only one of two swabs (nine from the craniofacial swab only, seven from the body swab only) in 16 snakes (47.1%).

Table 1. Prevalence by species. S/+ = positive with clinical signs, A/+ = positive without clinical signs.

Species	N	Positive	Prevalence (%)			
			For species	Overall	S/+	A/+
Eastern Wormsnake (<i>Carphophis amoenus amoenus</i>)	7	2	28.6	5.9	0	2
Northern Black Racer (<i>Coluber constrictor constrictor</i>)	3	2	66.7	5.9	2	0
Northern Ring-Necked Snake (<i>Diadophis punctatus edwardsii</i>)	1	1	100	2.9	0	1
Eastern Kingsnake (<i>Lampropeltis getula</i>)	1	1	100	2.9	1	0
Northern Watersnake (<i>Nerodia sipedon sipedon</i>)	26	19	73.1	55.9	15	4
Eastern Ratsnake (<i>Pantherophis alleghaniensis</i>)	10	7	70	20.6	5	2
Dekay's Brownsnake (<i>Storeria dekayi</i>)	2	1	50	2.9	1	0
Common Ribbonsnake (<i>Thamnophis saurita saurita</i>)	9	1	11.1	2.9	1	0
Eastern Gartersnake (<i>Thamnophis sirtalis sirtalis</i>)	2	0	0	0	0	0
Total or overall prevalence	61	34	-	55.7	25	9

Discussion

Although *Oo* has previously been documented in Maryland and Virginia (Guthrie et al. 2016; Tupper et al. 2018), this work is one of only two studies (see Guthrie et al. 2016) to investigate *Oo* in these states. In Maryland, observations of fungal dermatitis have been reported from the Smithsonian Environmental Research Center (SERC) since 2014 (Tupper et al. 2015), with *Oo* recently being confirmed as the etiological agent of a dermal infection in Northern Watersnake (Tupper et al. 2018). These results add four new species (Eastern Wormsnake, Northern Black Racer, Northern Ring-necked Snake, and Eastern Ratsnake) to the documented host range of this pathogen in Maryland, which previously included only Northern Watersnake (Tupper et al. 2018) and Timber Rattlesnake (*Crotalus horridus*; Tupper et al. 2019). In eastern Virginia, Guthrie et al. (2016) documented *Oo* in four species (all with clinical signs): Northern Watersnake ($n = 3$), Rainbow Snake (*Farancia erythrogramma*; $n = 1$), Northern Black Racer ($n = 2$), and Brown Watersnake (*Nerodia taxispilota*; $n = 2$). This study adds four new hosts to the list of *Oo* positive species occurring in Virginia: Eastern Kingsnake, Eastern Ratsnake, Dekay's Brownsnake, and Common Ribbonsnake.

The overall detection rate of 57.4% is among the highest reported (except see McKenzie et al. 2018) across the eastern and midwestern United States (Smeenk et al. 2016; Allender et al. 2016). The prevalence of *Oo* throughout these regions appears to be highly variable, with detection rates as low as 0% and 4.9% in Ohio and Michigan, respectively (Smeenk et al. 2016; Allender et al. 2016), and up to nearly 62% in eastern Kentucky (McKenzie et al. 2018). We interpret these rates cautiously, however, taking into consideration the variation in species sampled between studies. It is still unclear how susceptibility and severity of infection differ between species (Grisnik et al. 2018), but the composition of species sampled in this study may partly explain the overall prevalence and the relatively high proportion of *Oo* positive snakes that did not show clinical signs of the disease.

In this study, *Oo* was detected in eight of the nine species sampled, which was not surprising given that each of these species has previously tested positive for the pathogen in the eastern and mid-western United States (Lorch et al. 2016; Persons et al. 2017; Grisnik et al. 2018; McKenzie et al. 2018). However, the small sample sizes in certain species made it impossible to assess how each of these species actually influence the overall detection rate. *Ophidiomyces ophiodiicola* was found to be most prevalent in Northern Watersnake, with a detection rate of 73%. This species represented nearly 43% of the total sample and thus had a strong influence on overall prevalence (55.7%). Prior studies with similar proportions of aquatic species have also demonstrated relatively high *Oo* detection rates among Northern

Watersnakes and other species with aquatic affiliations. However, this trend in detection may partially reflect the habitat preferences of the pathogen (Lorch et al. 2016; McKenzie et al. 2018), rather than an inherent biological susceptibility to the pathogen. Additional work is needed to better understand susceptibility to the disease.

Variability in sampling methods between studies should also be considered when interpreting results (McCoy et al. 2017; Grisnik et al. 2018; Hileman et al. 2018; McKenzie et al. 2018). For instance, the number of sterile dry swab applicators used per snake has been shown to influence detectability of *Oo*, with the use of only one applicator greatly increasing the probability of obtaining false-negatives (Hileman et al. 2018). The results obtained here support this concept, with 47.1% of snakes testing positive for *Oo* in only one of two swabs.

Underestimation of the prevalence of *Oo* may also occur when diagnostic tests are limited only to snakes that present clinical manifestation of infection (see Guthrie et al. 2016). While clinical signs have been associated with a higher probability of PCR-positive results (Allender et al. 2016), studies have also demonstrated that anywhere from 6% (Bohuski et al. 2015) to 38% (Hileman et al. 2018) of snakes without clinical signs test positive for *Oo*. The data reported here support these studies, with 26.5% of *Oo* positive snakes in this sample showing no signs of infection. One possible explanation is that clinical signs may be subtle and overlooked during inspection, because a snake is either in the early stages of infection or effectively clearing the infection through repeated sheds (Lorch et al. 2016; Grisnik et al. 2018; Hileman et al. 2018). Detection without clinical signs may also reflect the absence of infection in a specimen altogether. *Ophidiomyces ophiodiicola* can persist as a saprobe in the soil, which can facilitate transmission and increase the likelihood of a snake encountering *Oo* (Allender et al. 2015; Lorch et al. 2016). The presence of *Oo* on the skin, however, does not necessarily indicate infection. Therefore, while swabbing can be an effective, low-cost, and minimally invasive method for detecting the pathogen, it cannot be used to infer or imply infection status.

Results from this study confirm that *Oo* is present and relatively prevalent in both Maryland and Virginia, and that the presence of *Oo* is more often accompanied by clinical manifestations consistent with ophidiomycosis than not. The geographic distribution and host range of the pathogen are still largely unknown (Burbrink et al. 2017), and ophidiomycosis may be more widely distributed than documented cases suggest (USGS 2018). Some have proposed that biased sampling methods may result in underestimations of prevalence within a population (Grisnik et al. 2018; Hileman et al. 2018). This potential for inaccurate assessments highlights the need for more standardized sampling efforts and diagnostic protocols. Based on the increasing number of reports of ophidiomycosis throughout the eastern United States, we suggest increased efforts to identify and monitor

Oo throughout the mid-Atlantic region. Additionally, enhanced biosecurity protocols should be implemented to limit disease transmission throughout the region.

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Lauren Fuchs is a Ph.D. student in Environmental Science and Policy at George Mason University (Fairfax, Virginia, USA). Lauren is also a graduate teaching assistant, and volunteer at the Smithsonian Environmental Research Center (Edgewater, Maryland, USA). Her main area of interest is in the diseases of herpetofauna.

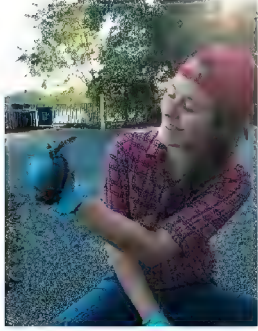


Todd Tupper is a Professor of Biology at Northern Virginia Community College (Alexandria, Virginia, USA), an affiliate faculty member at George Mason University (Fairfax, Virginia, USA), and a Visiting Researcher at the Smithsonian Environmental Research Center (Edgewater, Maryland, USA). Todd teaches general biology, zoology, and biostatistics. His areas of interest are biology education, and amphibian and reptile monitoring and conservation.

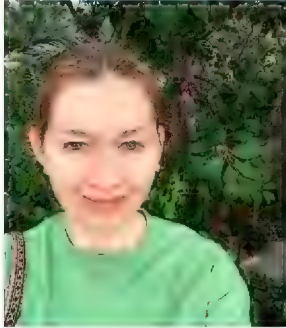


Robert Aguilar is a biologist at the Smithsonian Environmental Research Center (Edgewater, Maryland, USA). His areas of interest are quite varied and include fish and invertebrate ecology and phylogenetics, herpetological diseases, wildlife inventory and monitoring, and science education. Robert helps with the Maryland Biodiversity Project and supervises undergraduate researchers at the Smithsonian Environmental Research Center.

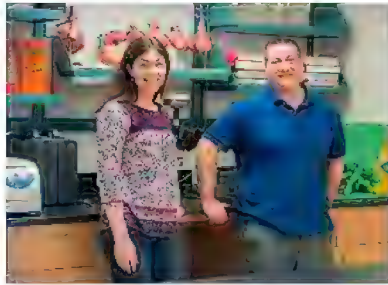
Ophidiomycosis in the mid-Atlantic USA



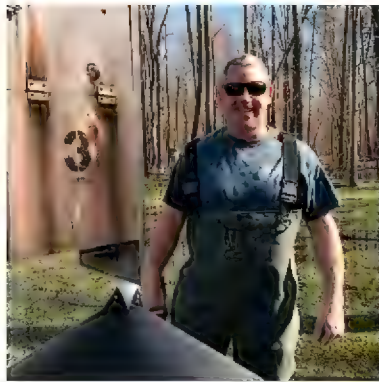
Eva Lorentz is an undergraduate senior in Biology at George Mason University (Fairfax, Virginia, USA) and a volunteer for the Smithsonian Environmental Research Center (Edgewater, Maryland, USA).



Christine Bozarth is an Associate Professor of Environmental Science at Northern Virginia Community College (Alexandria, Virginia, USA). Her areas of interest are in population genetics, wildlife inventory and monitoring, and science education.



David Fernandez is a Professor of Biology at Northern Virginia Community College (Alexandria, Virginia, USA). David teaches cell biology, general biology, and anatomy and physiology. His areas of interest are in hepatic cancers and wildlife disease. His areas of interest are in hepatocyte biology, tumor biology, and herpetological diseases.



David Lawlor is a biologist at Huntley Meadows Park in Alexandria, Virginia, USA. His areas of interest are in natural history, natural resource management, and science education. David also coordinates and oversees the wildlife research that occurs at Huntley Meadows.



Insights into the natural history of the endemic Harlequin Toad, *Atelopus laetissimus* Ruiz-Carranza, Ardila-Robayo, and Hernández-Camacho, 1994 (Anura: Bufonidae), in the Sierra Nevada de Santa Marta, Colombia

^{1,*}Hernán D. Granda-Rodríguez, ²Andrés Camilo Montes-Correa, ³Juan David Jiménez-Bolaño, ⁴Alberto J. Alaniz, ⁵Pedro E. Cattán, and ⁶Patricio Hernáez

¹Programa de Ingeniería Ambiental, Facultad de Ciencias Agropecuarias, Universidad de Cundinamarca, Facatativá, COLOMBIA ^{2,3}Grupo de Investigación en Manejo y Conservación de Fauna, Flora y Ecosistemas Estratégicos Neotropicales (MIKU), Universidad del Magdalena, Santa Marta, COLOMBIA ⁴Centro de Estudios en Ecología Espacial y Medio Ambiente, Ecogeografía, Santiago, CHILE ⁵Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, CHILE ⁶Centro de Estudios Marinos y Limnológicos, Facultad de Ciencias, Universidad de Tarapacá, Arica, CHILE

Abstract.—*Atelopus laetissimus* is a bufonid toad that inhabits the mountainous areas of the Sierra Nevada de Santa Marta (SNSM), Colombia. This species is endemic and endangered, so information about its ecology and distribution are crucial for the conservation of this toad. Here, the relative abundance, habitat and microhabitat uses, and vocalization of *A. laetissimus* are described from the San Lorenzo creek in the SNSM, as well as its potential distribution in the SNSM. To this end, 447 individuals were analyzed during several sampling trips from 2010 to 2012. Against expectations, population density was significantly higher in the stream than in the riparian forest. Overall, *A. laetissimus* used seven different diurnal microhabitats, with a high preference for leaf litter substrates and rocks. The rate of recaptures decreased linearly across the survey nights. Two types of vocalizations related to the advertisement call of *A. laetissimus* were recorded: a series of pulsed calls like a buzz and another short call, lacking pulses or partially pulsed. According to this analysis, the areas with higher habitat suitability for *A. laetissimus* were located principally in the northern and northwestern regions of the SNSM, in agreement with literature. Moreover, the data modeling indicated a significant increase in habitat loss from 2013 to 2017. The information presented here should be considered as a starting point for the conservation of this species.

Keywords. Advertisement call, amphibian decline, conservation, ecology, habitat loss, habitat suitability, home range, microhabitat selection, nocturnal site fidelity

Resumen.—*Atelopus laetissimus* es un sapo de la familia Bufonidae que habita las zonas montañosas de la Sierra Nevada de Santa Marta (SNSM), Colombia. Para esta especie endémica y en peligro, la información ecológica y de distribución es crucial para su conservación. En el presente trabajo describimos la abundancia relativa, usos de hábitat y microhábitat, y las vocalizaciones de *A. laetissimus*, así como su distribución potencial en la SNSM. Para esto, analizamos 477 individuos durante varios muestreos entre 2010 y 2012. Contra las expectativas, la densidad poblacional fue significativamente mayor en el lecho de la quebrada que en el bosque ribereño adyacente. En general, *A. laetissimus* utilizó siete microhábitats diurnos, con una alta preferencia por los sustratos de hojarasca y rocosos. La tasa de capturas decreció linealmente a lo largo de los muestreos nocturnos. Registramos dos tipos de vocalizaciones relacionadas con el llamado de anuncio de *A. laetissimus*. Una serie de llamados pulsados como zumbidos y otros más cortos, con pulsos ausentes o parcialmente pulsados. De acuerdo con nuestros análisis, las áreas con mayor idoneidad de hábitat se localizan en los sectores septentrionales y noroccidentales de la SNSM, lo que es concordante con la literatura. Además, el modelo construido indica un incremento significativo de la pérdida de hábitat entre 2013–2017. Esta información debe ser considerada como punto de partida para la conservación de esta especie.

Palabras clave. Ámbito doméstico (home range), conservación, declive de los anfibios, ecología, fidelidad de percha nocturna, idoneidad de hábitat, llamado de anuncio, pérdida de hábitat, selección de microhábitat

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Correspondence. ^{1,*}hernangrandar@gmail.com; ²andresc.montes@gmail.com; ³herpetos4@gmail.com; ⁴alberto.alaniz@ug.uchile.cl; ⁶pahernaiez@gmail.com

Introduction

The harlequin toads (Bufonidae: *Atelopus*) are small amphibians (<10 cm) which have aposematic coloration and predominantly diurnal activity periods (Lötters 1996). With a worldwide diversity of 96 described species (Frost 2019), the conservation of these amphibians has been seriously affected during the last decades due to the population decreases detected in a majority of species and the extinction of others, and currently 97.92% of the species of this genus are included in the IUCN Red List of Threatened Species, with one extinct species (Young et al. 2001; La Marca et al. 2005; Gascon et al. 2007; Tapia et al. 2017; IUCN 2019). Among the main risk factors for the decline of these populations are the loss of habitat, the introduction of exotic species which are potential predators and competitors, as well as deaths by pathogens (mainly by the chytrid fungus *Batrachochytrium dendrobatidis* [Bd]) and climate change (Lötters 2007; Catenazzi 2015; Barrio-Amorós and Abarca 2016; Valenzuela-Sanchez et al. 2017).

In general, information about the population biology and ecology of the different *Atelopus* species is relatively scarce. For example, the males of the species *Atelopus cruciger* (Lichtenstein and Martens, 1856) are known to remain longer in the streams than the females (Sexton 1958). But, on the other hand, studies conducted on *Atelopus carbonerensis* (Rivero, 1974 “1972”) in Venezuela, found that they remain almost all year in their habitat, except in the dry season when individuals migrate towards the streams for reproduction (Dole and Durant 1974). In the Variable Harlequin Toad of Costa Rica, *Atelopus varius* (Lichtenstein and Martens, 1856), both males and females are territorial and have fidelity for their reproduction sites. This indicates that even temporary alterations of the aggregation patterns of individuals between dry and rainy seasons, when they are more dispersed due to the increase in humidity (Crump 1988; Pounds and Crump 1989), results in an apparent decrease in detection (González-Maya et al. 2013). In Panama, the Golden Toad, *Atelopus zeteki* (Dunn, 1933) is concentrated in streams at the beginning of the breeding season, which occurs only during the transition between the rainy season and the dry season (Karraker et al. 2006). Another study determined site fidelity, habitat utilization, and range of households in the Suriname Toad, *Atelopus hoogmoedi* (Lescure, 1974) during the rainy season (Luger et al. 2009).

The Sierra Nevada of Santa Marta (SNSM) is a mountain massif located in the Caribbean region of Colombia. The particular conditions of isolation and vegetation of this mountainous system, which is not connected to the Andes, have led to a series of speciation processes in several groups of vertebrates, such as amphibians and reptiles (Ruthven 1922; Bernal-Carlo 1991; Sánchez-Pacheco et al. 2017), mammals (Alberico et al. 2000), and birds (Strewe and Navarro

2004). This applies to the harlequin toads (Bufonidae: *Atelopus* Duméril and Bibron, 1841), whose current diversity includes five endemic species for this region: *Atelopus arsyecue* Rueda-Almonacid, 1994, *Atelopus carrikeri* Ruthven, 1916, *Atelopus laetissimus* Ruiz-Carranza, Ardila-Robayo, and Hernández-Camacho, 1994, *Atelopus nahumae* Ruiz-Carranza, Ardila-Robayo, and Hernández-Camacho, 1994, and *Atelopus walkeri* Rivero, 1963. These species can be found between 800 and 4,500 m asl, and from the tropical moist forests to the páramos of the SNSM (Ruthven 1916; Rueda-Almonacid 1994; Ruiz-Carranza et al. 1994; Rueda-Almonacid et al. 2005).

This study examines some aspects of the natural history and ecology of the Harlequin Toad, *A. laetissimus*. Previous studies have shown that *A. laetissimus* inhabits streams and rivers in the mountainous areas of the northwestern sector of the SNSM (Granda-Rodríguez et al. 2008, 2012; Rueda-Solano et al. 2016a), and it is classified as Endangered (EN) by the IUCN (Granda et al. 2008). A recent study reported individuals infected by the chytrid fungus *Bd* (Flechas et al. 2017), the pathogen that has led to the decline and disappearance of many amphibian populations globally (Young et al. 2001; Catenazzi 2015). This study also includes an estimation of relative abundance, population density, microhabitat preference, spatial dynamics, and vocalization, in a population of *A. laetissimus* in the sector of San Lorenzo, Santa Marta, Colombia. Additionally, the potential distribution and patterns of habitat loss for this species during the 21st century were modeled, recognizing the great influence of habitat loss in recent decades on the extinction of many Neotropical species (Young et al. 2001; Marca et al. 2005; Lötters 2007).

Materials and Methods

Study Area

The study area corresponds to the San Lorenzo creek (11°6'56.21" N, 74°3'0.18" W, 2,100 m asl), an affluent of the upper basin of Gaira river, northwestern sector of the SNSM, Santa Marta district, Magdalena department (= state), Colombian Caribbean. The principal vegetation unit of this area corresponds to lower mountain humid forest (*sensu* Espinal and Montenegro 1963). According to Granda-Rodríguez et al. (2012), the annual averages of rainfall and temperature are 2,622 mm and 13.6 °C, respectively, and the climatic regime is unimodal bi-seasonal, with a dry period from December to March, and a rainy period from April to November.

Estimations of Relative Abundance and Population Density

Relative abundance (RA) was estimated from data collected in seven field campaigns conducted from 2010 to

2012 (October 2010; April, June, and December 2011; January and February 2012). Each fieldtrip had a duration of 12 days, with seven hours of daily work (0800–1200 h, 1800–2100 h). Individuals were detected through Visual Encounter Survey (Crump and Scott 1994), where two observers performed random walks. The sampling effort was 84 h per observer for each field campaign, reaching 558 h per observer in total. Relative abundance was calculated as the number of individuals/(h × observers), or ind/[h × obs] (Lips 1999). Sex was assigned by the size of individuals, assuming that females had a snout-vent length (SVL) > 40 mm and juveniles < 35 mm, and specimens within this range were considered as potential males. Sexual determination also considered the presence of eggs in the corporeal cavity noted through skin, calling behavior, and amplexant couples. Sex was determined in this way because the sexually dimorphic characters typically useful for population studies (La Marca et al. 1990 “1989;” Lampo et al. 2017) have not been established for *A. laetissimus*.

Population density was estimated through 40 permanent transects of 20 × 4 m (Jaeger 1994), with 20 located in the riparian forest and 20 in the stream. These transects were positioned parallel to the stream, separated by at least 20 m. Two observers walked along the transect counting the individuals only once. The medians of density obtained in each riparian forest and stream were compared using the Wilcoxon test for independent samples (*W*).

Habitat Selection and Dispersal Patterns

Testing the microhabitat preferences followed the concept of the third and fourth levels of habitat selection according to Johnson (1980), that indicate which components of the habitat are used and their proportions of use. For diurnal microhabitat, the substrate occupied by each individual was recorded according to the seven categories proposed by Granda-Rodríguez et al. (2008b): (I) rocks, (II) leaf litter, (III) fallen trunks, (IV) ferns, (V) leaves, (VI) bare floor, and (VII) others. The proportion of area for each microhabitat category was measured in 15 random plots of 4 m² located at the side of the stream. A Chi-squared test (χ^2) was performed, where the expected frequency was the number of individuals by substrate (*N*), while the observed frequency was calculated as the total number of individual (*N*) per area proportion of the substrate (%). Microhabitat selection was assumed when the proportion of substrate used was different from its availability, following the method of Molina-Zuluaga and Gutiérrez-Cárdenas (2007). To determine which substrates were selected by individuals, this analysis was repeated after deleting the categories most used or those that seemed to be used disproportionally to their availability (Molina-Zuluaga and Gutiérrez-Cárdenas 2007). In cases where significant differences in the second anal-

ysis were not found, the deleted category was considered to be preferred by the species.

To determine the nocturnal site fidelity, 60 specimens were marked with sub-epidermal alphanumeric tags (Visible Implant Alpha Tags, Northwestern Marine Technology Inc., 1.5 × 2.5. mm), and detected at night with a fluorescence lantern (Courtois et al. 2013). During 13 continuous nights (1900–2200 h, 36 h × obs), two observers looked for marked individuals to determine if they stayed in the same sites. The date, hour, location, and distance from the previous capture site were recorded for each recapture. The potential relationship between the number of individuals recaptured and the number of nights of survey was explored using a linear regression.

To describe the patterns of diurnal horizontal movement, each marked and recaptured specimen was spatially located in a Cartesian diagram consisting of a 50 m transect along the stream delimited by a reference point every 5 m (*y* axis), and the perpendicular distance of the specimen to the transect (*x* axis). Then, the distance from diurnal to nocturnal microhabitat was measured when possible. This was carried out in two sampling sessions of three days, with a three-day interval between them. The sampling times on the first day were 0800–1100 h and 1400–1600 h, on the second at 0600–0900 h and 1300–1500 h, and on the third at 0900–1200 h and 1500–1700 h. This sequence was repeated successively. Aggressive behavior observed during the survey was described following the terminology of Crump (1988).

Advertisement Call

On 30 January 2012, 270 seconds (s) of the advertisement call (*sensu* Wells 2007) of a male of *A. laetissimus* was recorded in San Lorenzo creek, at 1732 h, using a Sony (ICD-PX312) digital recorder. Although the advertisement call is not easy to define, we consider that the recorded calls belong to this functional category because they were emitted regularly by a solitary male *in situ* (without manipulation), who had no interactions with individuals of the same sex (which might indicate aggressive calls) or the opposite sex (which might indicate courtship calls). Air temperature and relative humidity at the recording moment were 12.3 °C and 75%, respectively. The traits of advertisement calls were quantified using the software PRAAT 6.0.13 for Windows (Broesma and Weenik 2007). The parameters of the advertisement call measured were: call duration (in seconds: s), number of pulses per call, pulse duration (s), interpulse interval (s), rate of pulses per second (pulses/s), frequency range (Hz), dominant frequency (Hz), and visible harmonics (Hz). Means and standard deviations (SD) were calculated for each call parameter. The terminology proposed and revised by Cocroft et al. (1990) was used for call types and by Köhler et al. (2017) for call parameters. Spectrograms and oscillograms were generated using the Seewave package (Sueur et al. 2008) in R environment (R Core Team 2018).

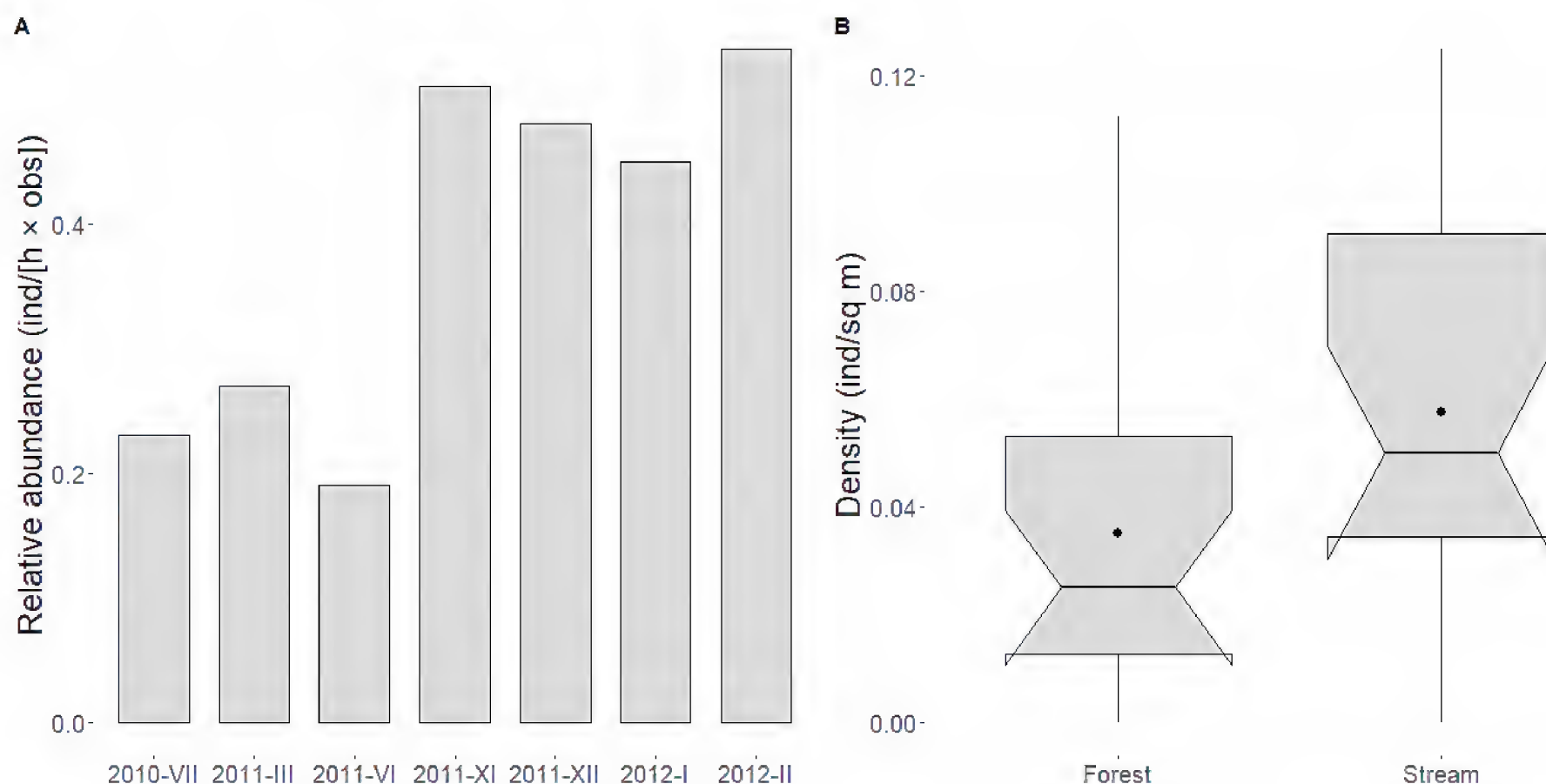


Fig. 1. (A) Temporal variation of relative abundance (ind/[h × obs]). (B) Population density (m²) per habitat of *Atelopus laetissimus*. Roman numerals represent the months of the surveys.

Potential Distribution and Habitat Loss

A species distribution model was performed, which predicts the habitat suitability with predictive algorithms integrated from environmental data and museum records (Phillips et al. 2017). The available records of *A. laetissimus* were compiled from Global Biodiversity Information Facility (GBIF), and the authors' own data, considering all known records from 1969 to 2017. Nineteen bioclimatic layers from Worldclim 2.0. (Fick and Hijmans 2017) plus altitude, human footprint (Venter et al. 2016), and solar radiation were used as predictor variables. First, data were explored through a preliminary model including all variables, with the technique of maximum entropy (MaxEnt software, 3.4.1, Phillips et al. 2017), considering that this algorithm is not hindered by a minimum number of occurrences. Variables with correlation indexes > 0.7 and lower contributions to the exploratory model were removed. To reduce the overfitting of the model, collinearity was determined with a Spearman correlation test. According to van Proosdij et al. (2016), the size of the background was considered in relation to the prevalence of the species to be modeled (< 25 localities), since this criterion generates acceptable results for species with restricted distributions.

A model of seven-fold bootstrap technique was performed, using 65% of data for training and 35% of data for testing (Puschendorf et al. 2008), considering the small number of locations that could be used (Elith et al. 2011). The average and standard deviation of the predicted suitability were used as a final model, and as a spatially explicit measure of the reliability of the prediction, respectively. The accuracy of the model was estimated using the metric of the area under the curve of the receiver operating characteristic (AUC, Elith et al. 2011).

Additionally, the distribution extent was calculated using the IUCN methodology (2019), based on the area of occupation (AOO) and extent of occurrence (EOO). The AOO was calculated as the intersection of the species occurrence with a square grid of 2 × 2 km, while EOO corresponded to the minimum convex polygon drawn on the peripheral localities of the distribution area. Both AOO and EOO were calculated using only records after 2010.

To determine habitat loss, the resulting map from the distribution model was overlapped with forest cover loss maps from 2000 to 2017. These forest cover loss maps were generated by Hansen et al. (2013), who monitored the changes of forest cover annually with a spatial resolution of 30 m. This product has shown important benefits in terms of its feasibility for evaluating the loss and fragmentation of habitat for forest specialist species (Alaniz et al. 2018; Carvajal et al. 2018). The cumulative and annual habitat losses were calculated for the potential distribution, AOO, and EOO. For the annual habitat loss, fourth order polynomial regressions were performed to test the trends of the multianual habitat loss. The AOO and EOO estimations, and their respective trends, allowed a suggested threat classification based on criterion B of IUCN (2019) Red List of Species.

Results

Relative Abundance and Population Density

A total of 447 individuals of *A. laetissimus* were recorded, distributed potentially among 428 males, 16 females, and three juveniles. The numbers of individuals per survey fluctuated from 32 to 90 (median ± interquartile range, 78 ± 44.5 individuals), with a general RA of 0.38 ind/(h × obs), and 0.21 to 0.54 ind/(h × obs) for each survey (Fig. 1a). *Atelopus laetissimus* showed a population

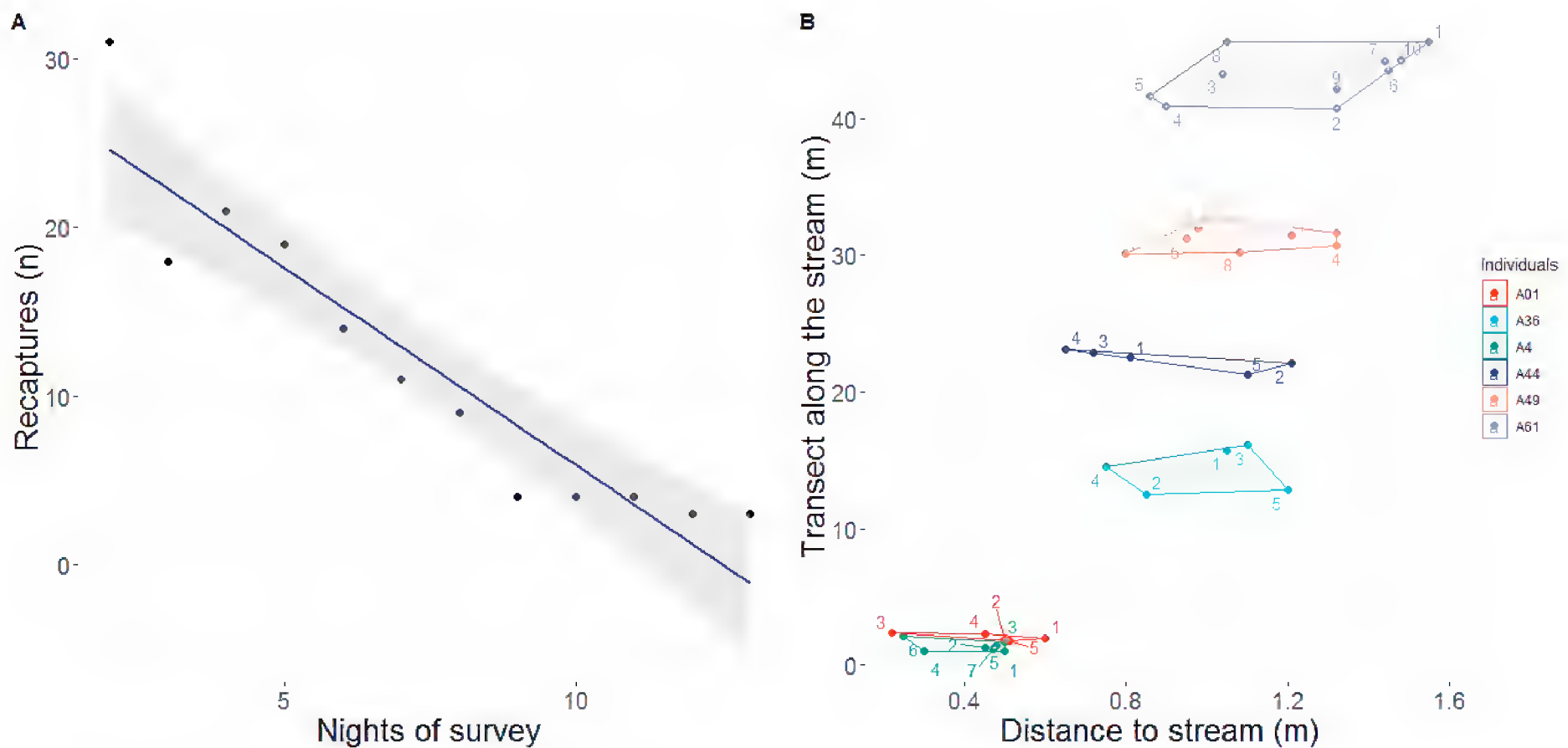


Fig. 2. Temporal variation of the number of recaptures (A) and movement patterns (B) of *Atelopus laetissimus*.

density from 0 to 0.13 ind/m² (0.04 ± 0.06 ind/m²). Population density was significantly higher (Wilcoxon test, $W = 277.5$, $p = 0.036$, Fig. 1b) in the stream ($0\text{--}0.12$ ind/m², 0.05 ± 0.07 ind/m²) than in the riparian forest ($0\text{--}0.11$ ind/m², 0.03 ± 0.05 ind/m²). The corresponding author will provide tables of raw data for individual specimens on request.

Habitat Selection and Dispersal Patterns

Atelopus laetissimus used seven different diurnal microhabitats, which were also used differentially regarding their availability ($\chi^2 = 120.121$, $df = 6$, $p < 0.001$). Although the leaf litter and rocks were the most used substrates (Table 1), significant differences in the use of microhabitat were still evident when these were removed from the analysis ($\chi^2 = 471.991$, $df = 4$, $p < 0.001$), suggesting that there is no preference for these substrates.

From 60 marked specimens, three (5%) to 31 (52%) were recaptured per night of survey. The recapture rate was higher than 30% until the fifth night, while after the ninth night it was reduced to less than 7%, showing a linear decrease across the sampling nights ($r^2 = 0.86$, $F_{1-10} = 69.99$, $p < 0.001$, Fig. 2a). The height of the nocturnal microhabitat ranged between 10 and 168 cm (mean $\pm$ SD, 73.03 ± 48.41 cm). The nocturnal site fidelity of *A. laetissimus* did not appear to be related to its height or to the SVL of specimens. Only six of the 60 tagged individuals were recaptured more than four times. These specimens showed an average home range of 0.35 ± 0.21 m² ($0.1\text{--}0.59$ m², Table 2, Fig. 2b), with an average horizontal displacement of 1.92 ± 0.82 m ($0.8\text{--}3$ m) relative to the nocturnal site. Most of the specimens were separated from each other by at least 5 m, but specimens A1 and A4 were very close to each other, so aggressive behavior between them could be observed. Specimen

A4 pounced and squashed specimen A1, then they began actively “wrestling.” This situation lasted about 120 seconds, until A1 fled. The “winner” male (A4) did not chase the male who left. The males did not emit vocalizations during the event.

Advertisement Call

Two types of vocalizations were recorded in a male *A. laetissimus*. The first call type corresponded to a short series of pulses like a buzz (mean $\pm$ SD, range, N; 27 ± 5.63 pulses, 7–33 pulses, 26 calls, Fig. 3a), with a duration of 0.41 ± 0.112 s ($0.111\text{--}0.805$ s, 26 calls). These pulsed calls showed modulated amplitude, where the amplitude increased along the call and decreased again at the last pulse. The pulse duration was 0.009 ± 0.006 s ($0.001\text{--}0.099$ s, 705 pulses), emitted at a rate of 67.55 ± 9.428 pulses/s ($32.298\text{--}76.167$ pulses/s, 26 calls). In most of these calls, the last pulse had a longer duration. The interpulse interval duration was 0.006 ± 0.004 s ($0.0001\text{--}0.097$ s, 652 interpulse intervals). These calls showed an ascending modulated frequency, although in some cases the frequency decreased notably at the last pulse. The frequency range was 1,287–8,558 Hz, while the dominant frequency was $1,921.433 \pm 114.391$ Hz ($1,480.95\text{--}2,155.55$ Hz, 631 pulses). In addition, the pulsed call of *A. laetissimus* showed three harmonics: first at 2,640.54–4,923 Hz, second at 3,661.56–6,644 Hz, and third at 5,771.95–8,558 Hz. The second type was a short call (Fig. 3b), with a duration of 0.06 ± 0.006 s ($0.05\text{--}0.07$, 12 calls). The short calls showed a variable structure, either unpulsed (Fig. 3b), partially pulsed (Fig. 3c), or pulsed (Fig. 3d). The short calls showed a low dominant frequency ($1,649.59 \pm 32.715$, $1,584.2\text{--}1,705.99$, 12 calls) and were produced irregularly, from a variable series, and alternated among vocalizations of

Table 1. Microhabitat selection by *Atelopus laetissimus* and χ^2 values for each substrate.

Substrate	Proportion of area	Individuals (<i>n</i>)	Expected frequency (<i>N</i> × proportion of area)	χ^2
Rocks	0.59	49	66.22	4.48
Leaf litter	0.28	28	31.19	0.33
Others	0.03	5	3.55	0.59
Ferns	0.01	3	0.75	6.81
Leaves	0.05	9	5.65	1.99
Bare floor	0.01	12	1.13	104.56
Fallen trunks	0.04	7	4.52	1.36
N	1	113	113	120.12

the first type (Fig. 3e), or within vocalizations of the first type (Fig. 3f).

Potential Distribution and Habitat Loss

The areas with higher habitat suitability for *A. laetissimus* are located mainly in the northern and northwestern sectors of the SNSM, which agrees with the occurrence localities. The model reached an AUC of 0.971 (± 0.011 , Fig. 4). The explanatory variables with the highest contributions to the suitability prediction were the average temperature of the coldest trimester (Bio 11), range of annual temperature (Bio 7), elevation, and human footprint. *Atelopus laetissimus* shows a maximum of suitability at 120 mm of rainfall at the coldest trimester; for annual temperature, it shows high suitability at middle ranges, rapidly decreasing at under 12 °C. The suitability shows a Gaussian trend regarding elevation, with a maximum at 2,000–3,000 m asl. Habitat suitability of *A. laetissimus* is inversely related with human footprint (Fig. 4).

The potential distribution area is 1,740.95 km², which corresponds to a continuous area in the northwestern sec-

tor of the SNSM, and a smaller and fragmented area in the northern sector. The AOO calculated by 13 plots was 54 km², while the EOO was 1,074.47 km². The habitat loss from 2000 to 2017 was larger in the southern sector of the SNSM, but was smaller in the northern and northwestern sectors of the massif. *Atelopus laetissimus* lost 1.48% of its habitat based on the potential distribution, 1.16% of its AOO, and 2.51% of its EOO (Fig. 5). A significant increasing trend in habitat loss was detected from 2013 to 2017, where the last year showed the greatest loss of potential habitat for the species. As *A. laetissimus* shows an EOO smaller than 5,000 km² (criteria B1b[i], B1b[ii], and B1b[iii]) and an AOO smaller than 500 km² (criteria B2b[i], B2b[ii], and B2b[iii]), these data reinforce its classification in the Endangered (EN) category.

Discussion

Relative Abundance and Density

The results of these surveys showed that *Atelopus laetissimus* is an easily detectable species in the northwestern

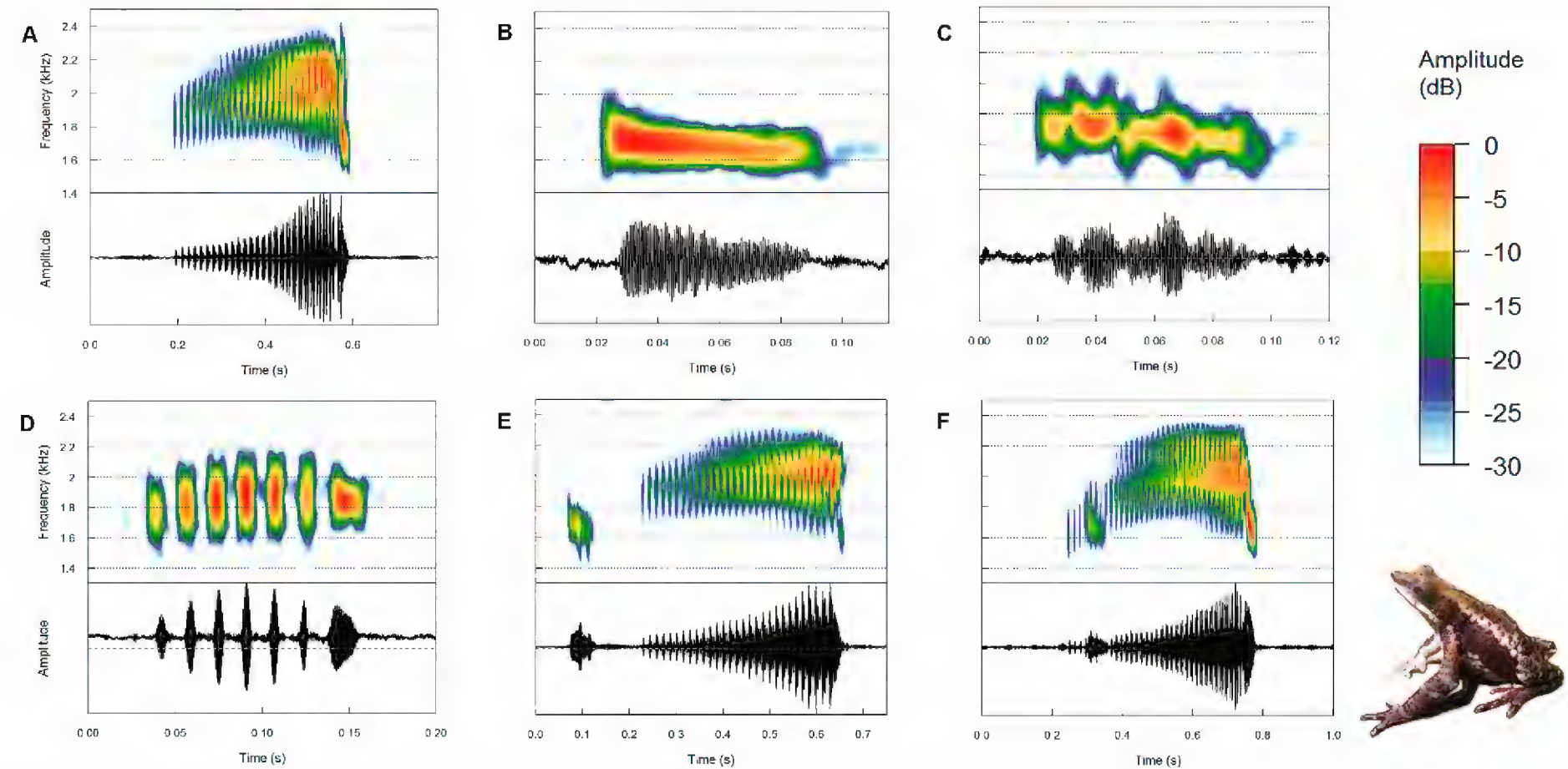


Figure 3. Acoustic repertoire of the advertisement call of *Atelopus laetissimus*. Conventional pulsed call (A), unpulsed short call (B), partially pulsed short call (C), pulsed short call (D), partially pulsed short call before pulsed call (E), and partially pulsed short call within pulsed call (F). The corresponding author will provide tables of raw data for individual specimens on request.

Table 2. Movement patterns of six *Atelopus laetissimus* individuals.

Specimen	Home range (m ²)	Recaptures (n)	Distance to nocturnal sites (m)
A4	0.11	7	2.5
A44	0.47	5	1.2
A49	0.33	8	3
A1	0.54	5	0.8
A61	0.1	10	1.8
A36	0.59	5	2.2
Mean	0.36	6.67	1.92
SE	0.21	2.07	0.82
Minimum	0.1	5	0.8
Maximum	0.59	10	3

sector of the SNSM. Several studies in this zone have reported more than 100 specimens in less than 150 h of survey effort (Granda-Rodríguez et al. 2012; Rocha-Úsuga et al. 2017; Rueda-Solano et al. 2016a). Nevertheless, at less than 2,100 m of elevation, the relative abundances recorded for the species were significantly lower (Car-

vajalino-Fernández et al. 2008, 2013; Granda-Rodríguez et al. 2012). In the model performed in this study, the most suitable habitat was in the altitudinal range between 2,000 and 3,000 m. It is possible that changes in the physical and structural characteristics of the habitat at lower altitudes contribute to the decrease in either the

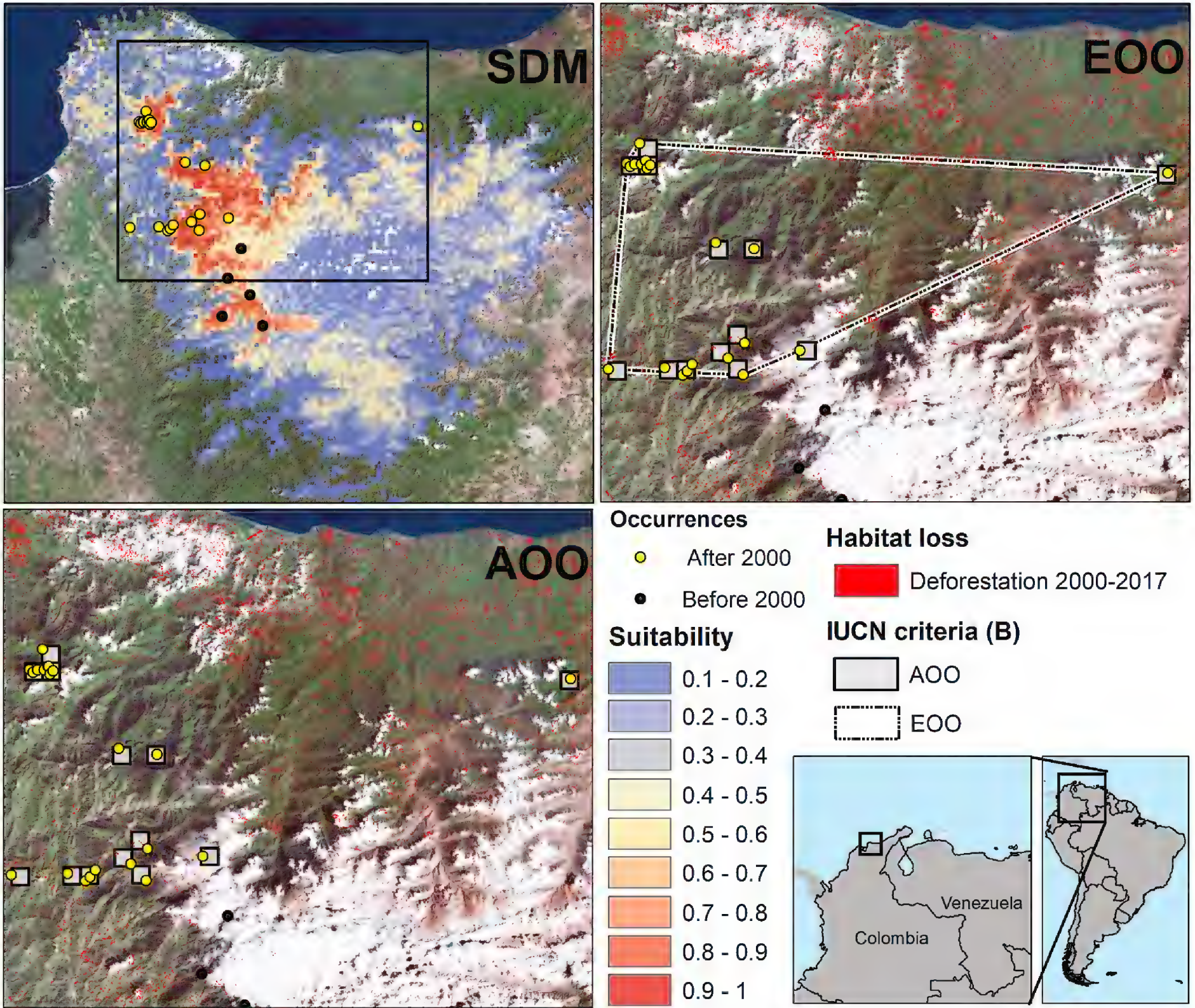


Fig. 4. Habitat suitability estimate (upper left panel), minimum convex polygon of extent of occurrence (EEO, upper right panel), and area of occupation (AOO, lower left panel) of *Atelopus laetissimus*. The total deforested area for the analyzed period and species occurrence locations are provided in red.

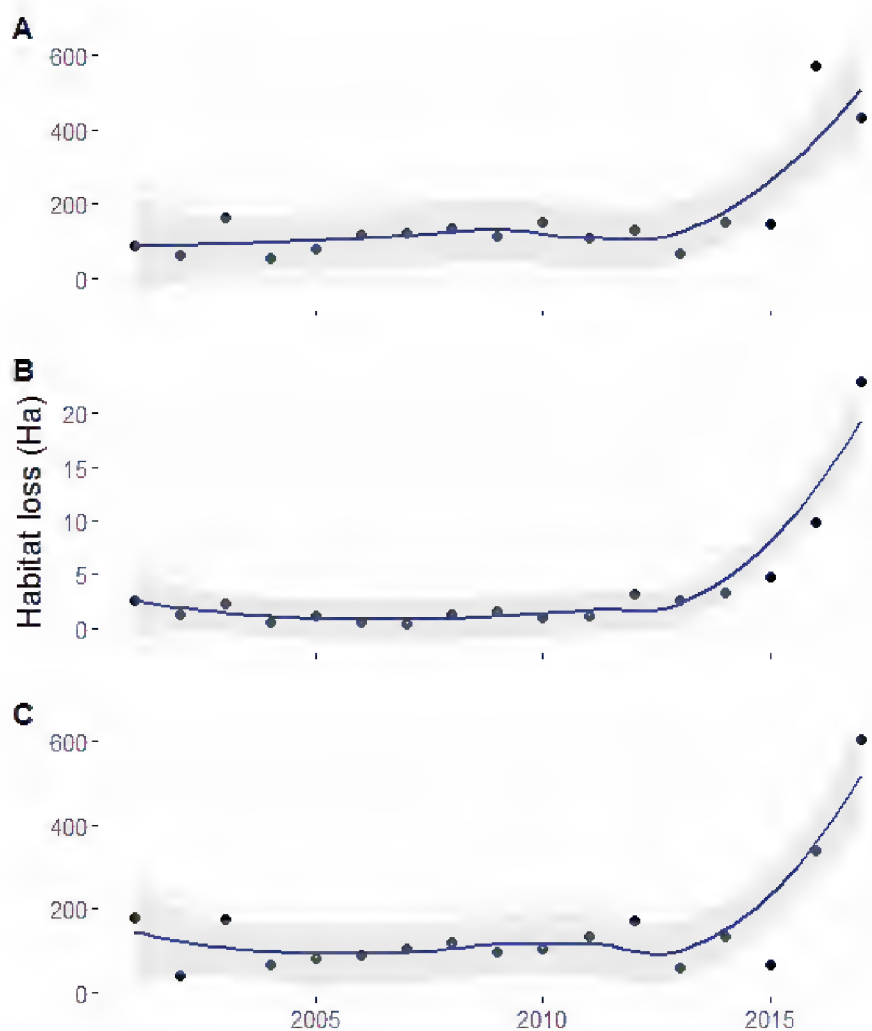


Fig. 5. Estimated annual habitat loss for *Atelopus laetissimus* in the last decade in the potential distribution (A), area of occurrence (AOO, B), and extent of occurrence (EOO, C).

occupation or detection of *A. laetissimus*.

Populations of *A. laetissimus* appear to be highly disproportionate in males. The first publication that mentioned this observation (Rocha-Úsuga et al. 2017) did not describe how the males were differentiated from the females. A sexual proportion that is biased to males can be related to differences in the mortality rate by sex. However, it is also possible that some individuals categorized as males corresponded to small females, especially since the sexuality was assumed based on size (e.g., Gómez-Hoyos et al. 2017). This is an important detail that has been addressed only rarely in population studies of *Atelopus* (Gómez-Hoyos et al. 2014; González-Maya et al. 2018), but it is important due to the conservation interest in the species of this genus.

Regarding population density, there is no previously published information for *A. laetissimus*. Since this study provides the first estimation of this population attribute, it is not possible to estimate variations among different populations of this species. However, comparing the population density of *A. laetissimus* obtained here with lowland species, the densities obtained with distance-based models for *Atelopus spurrelli* Boulenger, 1914 and *Atelopus elegans* (Boulenger, 1882) were slightly lower (0.03 and 0.01 ind/m², respectively, Gómez-Hoyos et al. 2014, 2017). On the other hand, the observed density of *Atelopus hoogmoedi* (0.47 ind/m², Luger et al. 2009) was higher than the mean density of *A. laetissimus* found in this study.

Some species, such as *Atelopus ignescens* (Cornalia, 1849) and *A. varius*, had dense populations before severe population declines, with reports of 0.025–0.75 ind/m²

and 0.065–0.755 ind/m², respectively (Ron et al. 2003; La Marca et al. 2005). *Atelopus cruciger* is one of the few species with information on population density after a decline, which was 0.005–0.057 ind/m² (Lampo et al. 2012). Populations of *A. cruciger* had a high prevalence of chytrid fungus, but remained stable because of the high recruitment rate of healthy individuals in the population (Lampo et al. 2017).

Habitat Selection and Movement Patterns

The differential use of several substrates by *A. laetissimus* has been previously reported, where leaf-litter and rocks were mainly used (Granda-Rodríguez et al. 2008b). The data reported here reinforce these findings, suggesting that this species selects the most available substrates. The structural complexity of the riparian forest occupied by *A. laetissimus* can influence its differential pattern of habitat use, as has been described for some anurans from southeastern Asia (Gillespie et al. 2004). Habitat selection allows organisms to avoid adverse environmental conditions, like extremely low temperatures (Navas 1996). Recently, *A. laetissimus* has been described as a thermoconforming species, showing a direct relationship between the temperature of substrate and the activity temperature (Rueda-Solano et al. 2016b). Therefore, the differential selection of substrates could be associated with some thermoregulatory strategy. The results of these surveys show that *A. laetissimus* exhibits relatively high nocturnal site fidelity, although the recapture rate was decreasing gradually, probably due to the manipulation of the specimens in each recapture. Recently, Rueda-Solano and Warketin (2016) reported that *A. laetissimus* use the nocturnal sites for predatory activities, guided by the vibration of the substrate (leaves and ferns), suggesting that the use of a nocturnal perch is not exclusively for rest.

Regarding the home range of *Atelopus*, some species such as *A. carbonerensis* and *A. hoogmoedi* possess a mean home range much larger than *A. laetissimus* (41 m² and 38.1 ± 17.7 m², respectively; Dole and Durant 1974; Luger et al. 2009), which could be the result of seasonal variation. The results here indicate that individuals of *A. laetissimus* can remain, at least for a short period, near to the stream defending their territories.

Advertisement Call

The pulsed call is the most commonly known vocalization in harlequin toads, being present in at least 17 species (Asquith and Altig 1989; Cocroft et al. 1990; Ibáñez et al. 1995; Jaslow 1979; Lescure 1981; Lötters et al. 1999, 2002; this study). The pulsed call of *A. laetissimus* consists of a short series of pulses (7–33 pulses) emitted rapidly, which is remarkably different from the pulsed calls of *A. barbotini* Lescure, 1981 (41–53 pulses per call, 30.35–33.97 pulses/s, 2,000–3,000 Hz; Lescure 1981),

A. flavescens Duméril and Bibron, 1841 (45–58 pulses per call, 29.76–34.78 pulses/s, 2,500–3,000 Hz; Lescure 1981), *A. franciscus* Lescure, 1974 (31–39 pulses per call, 22.97–23.78 pulses/s, 2,300–3,000 Hz; Lescure 1981), *A. hoogmoedi* (40–42 pulses per call, 33.61–35 pulses/s, 2,300–3,000 Hz; Lescure 1981), *A. spumarius* Cope, 1871 (20–37 pulses per call, 38.55–45.96 pulses/s, 3,600–4,400 Hz; Asquith and Altig 1987; Lescure 1981) or *A. reticulatus* Lötters, Haas, Schick, and Böhme, 2002 (27–32 pulses per call, 75–76 pulses/s, 3,282 Hz; Lötters et al. 2002) by having a higher number of pulses replicated more quickly at a lower dominant frequency. Likewise, it differs from the pulsed call of *A. zeteki* Dunn, 1933 (42–52 pulses per call, 115–146 pulses/s, 1,381–1,510 Hz; Cocroft et al. 1990), by having fewer pulses replicated more quickly at a higher dominant frequency.

Other species such as *A. cruciger* (84–99 pulses per call, 2,400–2,870 Hz; Cocroft et al. 1990), *A. limosus* Ibáñez, Jaramillo, and Solís, 1995 (31–45 pulses per call, 146.4–156.3 pulses/s, 2,600–2,800 Hz; Ibáñez et al. 1995), and *A. varius* (43–56 pulses per call, 119–123 pulses/s, 1,750–1,965 Hz; Cocroft et al. 1990) exhibit pulsed calls with higher numbers of pulses emitted at considerably faster rates than *A. laetissimus*. On the other hand, the structure of pulsed calls of *A. laetissimus* is very similar to the calls of *A. chiriquiensis* Shreve, 1936 (18–33 pulses per call, 59.5–82.3 pulses/s, 2,000–2,700 Hz; Jaslow 1979), *A. exiguus* (Boettger, 1892) [19–21 pulses per call, 2,150–2,700 Hz; Coloma et al. 2000], *A. minutulus* Ruiz-Carranza, Hernández-Camacho, and Ardila-Robayo, 1988, (14–21 pulses per call, 59.5–67.9 pulses/s, 2,700–3,150 Hz; Cocroft et al. 1990), *A. nicefori* Rivero, 1963 (21–24 pulses per call, 53.9–65.7 pulses/s, 2,630–2,871 Hz; Cocroft et al. 1990), *A. senex* Taylor, 1952 (30–34 pulses per call; Cocroft et al. 1990), and *A. tricolor* Boulenger, 1902 (16–19 pulses per call, 2,970–3,450 Hz; Lötters et al. 1999). Pulsed calls of these species also consist of shorter calls emitted at faster rates, but with higher frequencies than *A. laetissimus* in all cases.

The second type of vocalization (short calls) has been described for 12 species (Carvajalino-Fernández et al. 2017; Ibáñez et al. 1995; Jaslow 1979; Lötters et al. 1999, 2002; this study). This call is the more variable of the two in terms of structure, by the definitions proposed by Cocroft et al. (1990), which includes several vocalizations emitted in different social contexts. Both pulsed and short calls of *A. laetissimus* corresponded to the advertisement call context (*sensu* Wells 2007). This is probably the same situation for *A. varius*, whose short calls were obtained in the field, without apparent interaction among individuals (Cocroft et al. 1990). Short calls recorded in captivity for *A. cruciger* and *A. spumarius* also can be related to advertisement calls. This call is also reported in male-female interactions, in an amplexant couple of *A. zeteki*. Wells (2007) described this type of interaction as courtship calls. Encounter calls were re-

corded in a male-male aggressive interaction in *A. chiriquiensis* (Jaslow 1979). Nevertheless, most of the short calls described were release calls obtained at the moment of specimen manipulation (*A. chiriquiensis*, Jaslow 1979; *A. limosus*, Ibáñez et al. 1995; *A. nahumae*, Carvajalino-Fernández et al. 2017; *A. peruensis* and *A. tricolor*, Lötters et al. 1999). In addition, short calls of *A. tricolor* cannot be included in any of these categories, since the context of the recording was not described clearly (Lötters et al. 2002). Previously, the role of vocalizations in the communication of the genus *Atelopus* has been distorted by the absence of several elements of the auditory apparatus (McDiarmid 1971) and the conspicuousness of its visual communication (Jaslow 1979; Crump 1988). Nevertheless, the complexity and diversity of vocalizations described and reviewed here suggests that their roles in communication may be underestimated.

Potential Distribution and Habitat Loss

According to IUCN SSC Amphibian Specialist Group (2014), *A. laetissimus* is a species restricted to the moist low montane forest life zone, at altitudes between 1,500–2,880 and an area of 797 km². In this study, localities are reported between 900 and 2,880 m asl and an EOO of 1,074 km², higher than that reported by IUCN. Despite this increase in the distribution of the species which could be associated with new samplings, we recommend its status of Endangered (EN) be maintained. The niche model of *A. laetissimus* suggests that the potential distribution is restricted to forests in humid zones at the northwestern and northern flanks of the SNSM. The results show that the AOO of the species may not exceed 52 km². The analysis by the model suggested the environmental layers that most influenced the distribution of the species are the average temperature of the coldest quarter, the elevation, and the human footprint. In the case of amphibians, important influences of the climate on their distribution have been described, therefore, factors such as climate change could significantly alter populations of *A. laetissimus*. Among the potential changes are alterations of the precipitation and temperature regimes, resulting in an increase in the annual temperature ranges and thus affecting habitat quality and the availability of specific resources for *A. laetissimus* (Zhang and Yan 2014). On the other hand, human influence generates a very marked negative effect on this type of species in terms of degradation and loss of habitat (Grant et al. 2016).

The model performed here represents the first empirical estimation of the distribution of this species based on distribution modeling, and it also uses the largest compilation of localities. Additionally, recent samples in areas with high suitability predicted by the model support the reliability of the prediction (Rueda and Warkentin 2016). In any case, the predictions of these models should be interpreted with caution and they should be considered as a first approximation to the real distribution of the species,

helping to focus the sampling efforts in order to further adjust and refine the predictions of the distribution of this species in the future.

Although the habitat loss identified does not represent a high percentage in relation to the total habitat of the species, the trends showed a significant increase in loss in recent years (Ribeiro et al. 2018). However, considering that Hansen et al. (2013) does not differentiate between types of vegetation (natural and exotic plantations), the results could vary. This is important since *A. laetissimus* has scarcely been associated with exotic plantations of *Pinus* spp., or in streams associated with this type of coverage in the locality of San Lorenzo (Granda-Rodríguez et al. 2012). In addition, it is also not known how coffee and avocado plantations can affect this species, since in the middle- and upper-part of the distribution large areas of these crops are present (Fundación Pro-Sierra Nevada de Santa Marta 2000). It is necessary to carry out studies at smaller scales in the distribution area of *A. laetissimus*, allowing the identification of the landscape dynamics of forest patch isolations, connectivity, and the different elements that may have negative consequences for this species (Palmeirim et al. 2018). In this sense, remaining remnants of forest become important for the maintenance of *Atelopus laetissimus*, as well as other endemic species of the SNSM. However, in the southern sector of the SNSM there is high fragmentation and habitat degradation; and this site is (was?) inhabited by *A. arsyecue* and *A. walker*, species that have not been seen in the field for more than 20 years, providing possible evidence for the risk of disappearance of this type of toad.

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Hernán Granda Rodríguez is a Biologist from the University of Magdalena, Santa Marta, Colombia, who obtained his Master's degree in Wild Areas and Nature Conservation from the University of Chile, Santiago, Chile. Hernán is currently a professor in the environmental engineering program at the University of Cundinamarca, Cundinamarca, Colombia. His research interests focus on the conservation of amphibians and reptiles, from the perspectives of both biological issues and social issues, such as the public's perception of herpetofauna.



Andrés Camilo Montes-Correa is a Colombian young biologist and herpetologist (in training) at the Universidad del Magdalena (Santa Marta, Colombia). His research interests include the taxonomy, systematics, and ecology of the Colombian herpetofauna, especially lizards and freshwater turtles. Andrés has conducted investigations on habitat use by freshwater turtles of the Colombian Llanos (for his B.Sc. thesis); ecology, taxonomy, and distribution of small geckos and cryptic dendrobatid frogs; and synecological studies of herpetofauna. His current project is on the taxonomy, ecology, distribution, and conservation of *Lepidoblepharis miyatai*, a small and endangered gecko endemic to the coastal mountains (Sierra Nevada de Santa Marta) of the Colombian Caribbean.



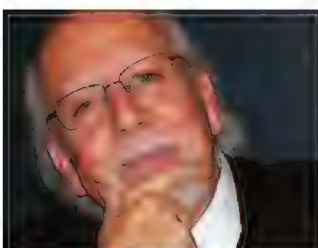
Juan David Jiménez-Bolaño is a wildlife photographer and has a B.Sc. in Biology from the University of Magdalena (Santa Marta, Magdalena, Colombia). Juan's work has focused mainly on the ecological and diversity patterns of Neotropical herpetofauna. Currently, Juan is interested in studying diversity patterns in high Andean environments.



Alberto J. Alaniz, Geographer, B.Sc., M.Sc. His work is focused on ecological modelling applied to biological conservation and epidemiology, and his recent research has been on the development of spatially explicit infection risk models for Zika virus at global and local scales. Currently, Alberto is a researcher in the Ecology and Conservation Lab in the Technological Faculty at the University of Chile, and he is a founding member of the Center for Spatial Ecology and Environment (Ecogeografía), located in the city of Santiago, Chile.



Patricio Hernáez is a Marine Biologist by training at the Universidad Arturo Prat (Chile), with a Master's degree in Biology from the Universidad de Costa Rica (Costa Rica), and a Ph.D. in Biological Sciences from the Universidade de São Paulo (Brazil). His research focus is divided into three main lines: (i) Taxonomy of recent groups with an emphasis on Crustacea, (ii) Population dynamics, and (iii) Fisheries. Patricio is currently a researcher associated with the Centro de Investigaciones Marinas y Limnológicas of the Universidad de Tarapacá, Chile, and the Grupo de Pesquisa em Biologia de Crustáceos of the Universidade Estadual Paulista 'Julio de Mesquita,' Brazil.



Pedro E. Cattán has a D.V.M. and a Ph.D. in Biological Sciences from the University of Chile, and he was the Chairman of the Department of Biological Sciences of the University of Chile for 10 years. Pedro has developed two main lines of research, one on the study of populations in wild vertebrates and the other on disease ecology at the wildlife level. He currently directs projects on the vectors and wild reservoirs of Chagas disease.



Do growth rate and survival differ between undisturbed and disturbed environments for *Sceloporus spinosus* Wiegmann, 1828 (Squamata: Phrynosomatidae) from Oaxaca, Mexico?

¹Carlos A. Torres Barragán, ²Uriel Hernández Salinas, and ^{3,*}Aurelio Ramírez-Bautista

¹Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Unidad Oaxaca, Hornos No. 1003, Col. Noche Buena, Santa Cruz Xoxocotlán, Oaxaca, Oaxaca 71230, MEXICO ²Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Unidad Durango, Calle Sigma 119 Fraccionamiento 20 de Noviembre II, Durango, Durango 34220, MEXICO ³Laboratorio de Ecología de Poblaciones, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Km 4.5 carretera Pachuca-Tulancingo, 42184, Mineral de La Reforma, Hidalgo, MEXICO

Abstract.—Demography is intimately related to the evolution of the life history of a species, since it describes the patterns of variation in the growth, maturation, reproduction, and survival of an organism through populations, species, and environments. In this study the growth, survivorship, and population structure were evaluated for an oviparous lizard, *Sceloporus spinosus* from two sites, a relatively undisturbed area (UA) and a disturbed area (DA; zone of land-use change) within the Natural Protected Area Yagul of southern Oaxaca, Mexico. The results showed different relative densities between seasons (higher during the wet season than the dry season), but not between populations. Males and females from the UA and DA showed similar growth rate patterns, and both sexes reached sexual maturity at a similar body size. The highest survival rates and recapture probabilities were found in the UA; however, males from both populations showed higher survival rates than females. Overall, this study suggests that land-use changes do not seem to cause wide variation in the analyzed demographic characteristics of this species. This work describes and quantifies demographic effects on some life history characteristics of a species endemic to Mexico. We argue for the need to analyze and compare many capture-recapture data for a species between locations in order to obtain a better assessment of the variation in the life history characteristics analyzed.

Keywords. Age class, Cormack-Jolly-Seber model, demography, density, natural protected area, toe-clipping method

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Introduction

The variation in life history characteristics of lizards is considered to be an outcome of phenotypic plasticity driven by changing environmental conditions (Stearns 1992; Adolph and Porter 1996). Studies investigating demographic parameters (e.g., density, sex ratio, natality, growth rate, age classes) and life history (e.g., SVL at sexual maturity, survival, reproduction, fecundity) in lizards have shown that different life strategies (e.g., growth rate, survival) in these vertebrates have evolved due to environmental changes caused by habitat loss and by changes in land use (Dunham 1982; Stearns 1992). This implies that the populations of any species may evolve different life history strategies according to the

characteristics of the environments that they inhabit (e.g., pristine or fragmented). Therefore, the conservation priority of populations of a widely distributed species, is to assess the tolerance of their life history characteristics to certain environmental factors (precipitation, humidity, radiation, pollution, deforestation, and others) that occur throughout the distribution of the species; therefore, this is a feasible method to test for changes in their fitness (Walkup et al. 2017). Evolution within and across species that inhabit fluctuating environments has resulted in changes of their life history strategies, such as size and age at sexual maturity, fecundity (clutch size), growth rate, and survival (Stearns 1992); and these changes have been found within different populations of a single species that is widely distributed (Dunham 1982; Cruz

Correspondence. ¹augusto.torres007@gmail.com, ²uherndez3@gmail.com, ^{3,*}ramibautistaa@gmail.com

et al. 2014; Pérez-Mendoza et al. 2014; Cruz-Elizalde and Ramírez-Bautista 2016). These variations in life history characteristics have been documented in several species that inhabit environments with different degrees of disturbance (Cruz et al. 2014; Cruz-Elizalde and Ramírez-Bautista 2016; Walkup et al. 2017). However, strong fragmentation of habitats by land use change, pollution, and global warming have adverse effects on these strategies, that consequently lead to population decline at the local level (Sinervo et al. 2010).

Due to concerns regarding the effects of environmental disturbances, some researchers have generated conservation models for various biological groups (e.g., birds, Escalante et al. 1998; mammals, Ceballos and Oliva 2005) that include significant amounts of information on the natural history of individual species. For example, Sinervo et al. (2010) described patterns of species decline and extinction in a diverse assemblage of lizard species of genus *Sceloporus* under thermoregulatory stress induced by global warming. Chávez (2011) and Calderón-Mandujano (2011) noted that land use change is another factor that has resulted in high levels of population decimation and extinction among lizards and amphibians. However, each species responds in different ways according to the pressures of their local environment (Tews et al. 2004; Suazo-Ortuño et al. 2007). This pattern has been documented in several wide-ranging species that occur in relatively pristine habitats as well as sites that are subject to varying degrees of disturbance (e.g., *Sceloporus grammicus*, Pérez-Mendoza et al. 2014; *S. minor*, García-Rosales et al. 2017; and *S. variabilis*, Cruz-Elizalde and Ramírez-Bautista 2016). Therefore, considering these factors, herein, the effects of a pristine and a disturbed habitat on some life history characteristics were evaluated in two populations of *Sceloporus spinosus* (Eastern Spiny Lizard) in southeastern Mexico.

Sceloporus spinosus is a species endemic to Mexico, and adults are of medium body size for the genus (120 mm snout-vent length, SVL; Ramírez-Bautista et al. 2014). The scales of the body are strongly keeled and mucronate. This species feeds on insects and other invertebrates, and it is oviparous with a clutch size of eight to 31 eggs and a mean of 18.5 (Valdéz-González and Ramírez-Bautista 2002). This lizard is found from Durango to Oaxaca, and inhabits arboreal and saxicolous landscapes (Torres-Barragán 2015) in both temperate and semiarid regions, at an elevation range from 1,900 to 2,700 m (Canseco-Márquez and Gutiérrez-Mayén 2010). In Yagul Natural Protected Area (NPA), this species is distributed in a mountain range with elevations from 1,600 to 2,000 m. So far, there is limited information regarding demographic aspects of this species in pristine and disturbed areas such as Yagul NPA. In this framework, the goal of this study was to compare and assess key demographic characteristics, such as density, growth, survival, and population structure of *S. spinosus* in two

contrasting environments, in Oaxaca, Mexico. Therefore, considering that land use change promotes variation in demographic characteristics and life histories in various vertebrate groups (Adolph and Porter 1996; Flatt and Heyland 2011), these demographic characteristics were expected to differ as a function of the environments where each population of *Sceloporus spinosus* occurred.

Materials and Methods

Study Area

This study was carried out at two sites in Yagul Natural Protected Area in the municipality of Tlacolula de Matamoros, Oaxaca, Mexico (Fig. 1). The municipality encompasses 1,076 ha, ranging in elevation from 600 to 2,500 m. The vegetation at the site is represented by tropical dry forest; however, much of it has been replaced by crops and grazing areas. The climate is semi-warm, with temperatures ranging from 16 °C to 26 °C, and mean annual precipitation from 400 to 800 mm (INEGI 2005).

Two sites of 1 ha each were chosen for this study. The first site was considered the undisturbed area (UA; 16.957922 N, -96.429953 W; 1,800 m), with a vegetation cover of 83% and an arboreal density of 697 individual trees/ha. This cover includes 80% tropical dry forest, 10% flood zone, 3% reedbed (*Arundo donax*), and 2% surface without vegetation (Torres-Barragán 2015). The second site was a disturbed area (DA; 16.959617 N, -96.450633 W; 1,652 m; Fig. 1). This site is an open area with agave plant cultivation and extensive grazing areas; canopy cover is 1% with an arboreal density of eight individual trees/ha; 50% of the land is used for cultivation of agave plants (*Agave angustifolia*), 20% for induced pasture, 10% for living fences (*Prosopis*, *Yucca*, *Celtis*, *Acacia*, *Opuntia*, and *Schinus*), and 20% of the surface has no vegetation (exposed floor; Torres-Barragán 2015).

Data Collection

Twelve sampling events were conducted at each site (UA and DA) from January 2014 to January 2015. Each sampling event was carried out over a single three-day period in each month at each of the two sites (for a total of six sampling days per month), with a sampling effort of three people from 0900–1800 h.

The method of mark-recapture of Lemos-Espinal and Ballinger (1995) and Ramírez-Bautista (1995) was used in this study. This method consisted of ectomization of phalanges (e.g., toe-clipping), a permanent marking technique that makes it possible to recognize every previously marked individual during each subsequent sampling event. Toe-clipping is commonly used to follow cohorts of lizard populations (Dunham 1978; Tinkle 1961, 1969). More recently, Guimaraes et al. (2014) and Olivera-Tlahuel et al. (2017) expressed some concern when using this method due to observed effects on the

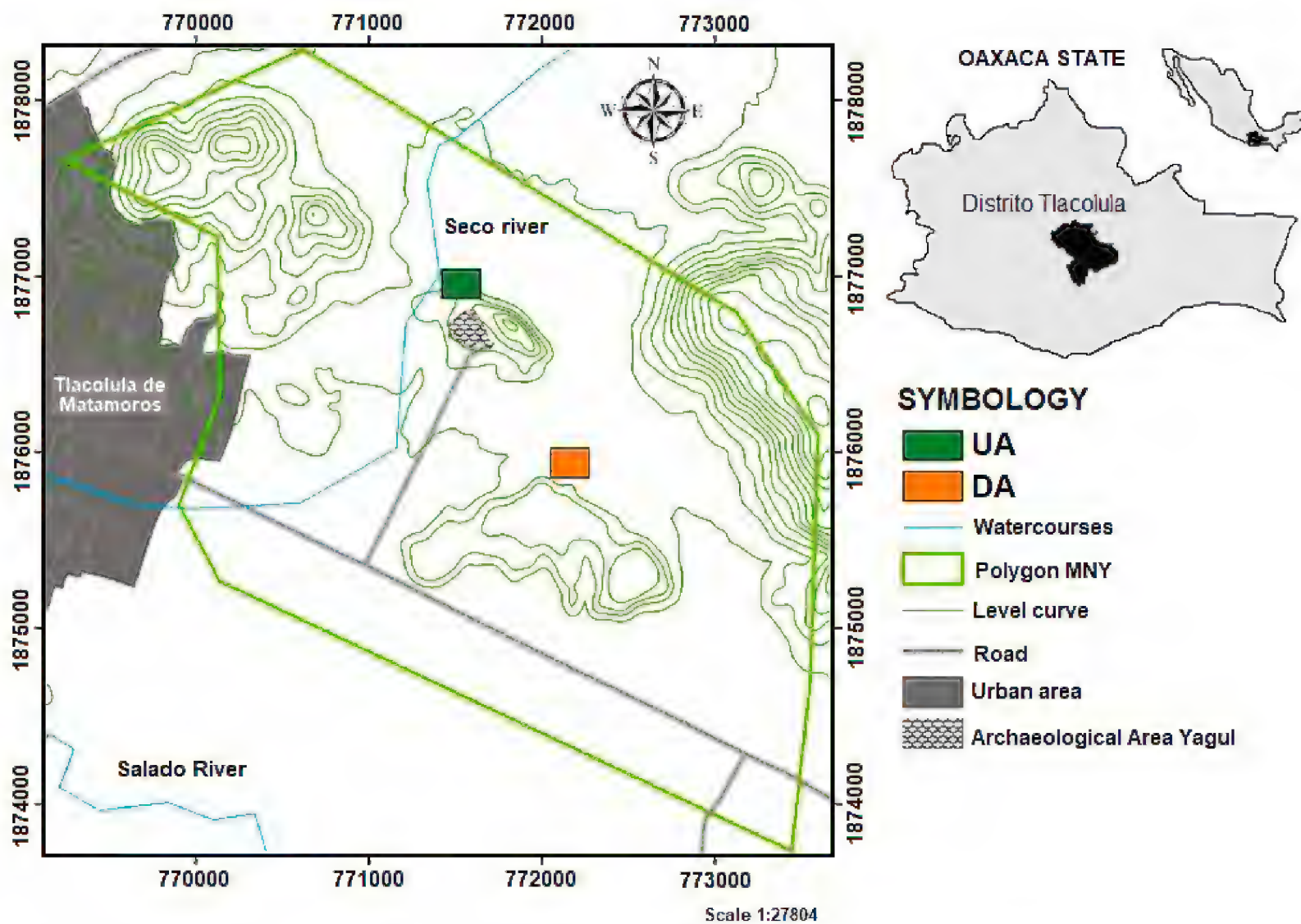


Fig. 1. Map of the study area. The green polygon depicts Yagul Natural Protected Area, including the two sampling sites (UA = undisturbed area; DA = disturbed area, land use change).

behavior and health of some lizard species, and therefore, on survival. Although toe-clipping could affect survival, lizards at both sites were toe-clipped similarly, so any negative bias in survival estimates should apply equally to both sites. The SVL of each lizard was measured with a digital caliper (to the nearest 0.01 mm), and body mass with a balance (± 0.01 g).

Relative Density and Population Structure

Based on the number of captured and recaptured individuals from both populations during the study, the relative density of each population was determined using the equation, $N = M/R$, where N = number of unknown individuals in the population; M = the number of marked individuals; and R = the number of recaptured individuals/surface area. To determine the population structure for each site, size classes (SVL) were determined based on those used by Leyte-Manrique et al. (2017) with *Sceloporus grammicus*. These authors related the SVL of each age category based on anatomical traits, yielding classification categories of: offspring ($SVL \leq 48$ mm), juveniles (49–69 mm), and adults (females and males ≥ 70 mm). Females were considered to be adults if they contained eggs in the oviduct, which were identified by palpation of the ventral region (Galán 1997). Whereas males were considered to be adults when they showed the bulky tail base indicative of sperm production (Lozano et al. 2014). Relative densities of lizards were compared

between locations and seasons by means of a Student's t -test (Zar 2014).

Growth Rate

Growth rates were assessed for males and females from each population by considering only those lizards with recapture intervals greater than 30 and less than 100 days. Therefore, growth rate was estimated with the formula: $GR = (SVL_2 - SVL_1)/\text{days}$, where growth rate (GR) is the difference in recorded SVL between the last recapture (SVL_2) and first capture (SVL_1) divided by the number of days that had elapsed (Dunham 1978; Zamora-Abrego et al. 2012). Then, nonlinear regression models of Von Bertalanffy, logistic by body size (SVL), and logistic by body mass were used (Dunham 1978); and growth rates for both sexes and populations were compared. The first model (Von Bertalanffy) describes a pattern in which smaller individuals (in SVL) show faster growth rates than larger ones (Dunham 1978; Zamora-Abrego et al. 2012). In contrast, the logistic models predict that individuals smaller in SVL will grow moderately faster to reach intermediate sizes, and after reaching their maximum growth rate, that rate will decrease in a non-linear direction as size increases (Dunham 1978; Schoener and Schoener 1978; Zamora-Abrego et al. 2012). The difference between the two models is that the maximum growth rate attained under a logistic by length (SVL) model is observed at early ages, while maximum growth rate under a logistic by body mass model will

be observed at later ages (Dunham 1978). Detailed descriptions of each model can be reviewed in Dunham (1978) and Schoener and Schoener (1978). Selection of the best model was based on the best fit to the observed growth rates for both sexes, chosen by the lowest value of the residual mean square (RMS) and highest values of coefficients of determination or correlation (R^2 ; Dunham 1978; Schoener and Schoener 1978).

Once selected, the models were developed with confidence intervals following Schoener and Schoener (1978) for the growth parameter (r), and the asymptotic (A_1) was calculated by the formula:

$$\theta_j - \sqrt{kF_{1-\alpha}(k, N-k)S_j^2} \leq \beta_j \leq \theta_j + \sqrt{kF_{1-\alpha}(k, N-k)S_j^2}$$

where β_j is the adjusted parameter j , θ_j is the parameter estimated β_j , S_j is the asymptotic standard deviation of β_j , $F_{1-\alpha}(k, N-k)$ is the value $F_{1-\alpha}$ of a tail with k and $N - k$ degrees of freedom, N is sample size, and k is the number of adjusted parameters. These confidence intervals provide maximum reliability for each parameter (r and A_1) and are considered significantly different between sexes if they do not overlap (Schoener and Schoener 1978). On the other hand, the residuals of the growth rate (removal of effect size) were used to determine by two-way ANOVA if there were differences in the patterns of growth rates between factors (sexes and populations). The residuals are the result of the relationship between the SVL and the growth rate under the model with the best fit (Schoener and Schoener 1978). Finally, based on the values of the growth parameter (r) and asymptotic (A_1) obtained from the best-fit model, together with the average values of SVL of offsprings at hatching ($L_0 = 42$ mm in SVL), the ages in days were determined for both males and females at which they reach sexual maturity (Dunham 1978; Schoener and Schoener 1978; Zamora-Abrego et al. 2012). The growth models for both sexes and populations were developed with Statistica program, version 7.0.

Estimates of the Survival Models

Captures and recaptures of marked individuals allowed estimates of demographic parameters, such as survival (ϕ) and recapture (p); and both parameters were estimated from different models that represent distinct biological hypotheses of survival (Lebreton et al. 1992). Both ϕ and p can be constant (c) over time or vary as a function of time (t) and between sex, and for their assessment a general model was considered which allowed the determination of whether survival rates and recaptures were different between sexes and populations. This model is: ϕ (groups [males and females in two populations = four groups]*time) p (groups*time), and it calculates the probability that survival and rate of recapture are different between groups over different periods of time.

For developing the different models based on encounter histories of each individual (e.g., 100101), zero represents sampling when a lizard was not seen in the area, while 1 represents those sampling events when lizards were marked and recaptured (Lebreton et al. 1992). Encounter histories were analyzed and modeled with software Mark 6.0 (White and Burnham 1999) using the subprogram “only recaptures” with the goal of obtaining estimates of survival and recapture rates grounded in the techniques of maximum likelihood under the model developed by Cormack-Jolly-Seber (Lebreton et al. 1992). The model that showed the best fit to the capture-recapture data was the one with the lower Akaike information criterion (AIC) value; however, when there was a difference of 2 between AIC values of the two models, both models were assumed to have approximately the same fit to the data (Burnham and Anderson 2002). Survival and recapture results are represented with confidence intervals of 95%.

Results

Relative Density and Population Structure

In the surveys, 271 individuals were marked across both sites (UA = 149 and DA = 122); of these, 113 were recaptured (73 in UA and 40 in DA; Table 1). In some cases, several individuals were captured as juveniles and then recaptured as adults; but most of the recaptures were adult males and females (Table 1). The relative density between seasons was different ($t_{1,11} = -2.023$, $P = 0.05$; wet: 6.95 ± 0.41 [4.92–10.07]; dry: 9.70 ± 1.29 [2.68–17.21]), but not between populations ($t_{1,11} = -0.116$, $P = 0.98$; UA: 8.33 ± 0.97 [5.68–16.11]; DA: 8.33 ± 1.11 [3.28–17.21]). The relative density for UA (both sexes and all age classes) was 149 individuals/ha, whereas for DA it was 122 individuals/ha. In UA, offspring emerged from July to December, but the peak hatching period was in September; juveniles were recorded from October to April, but the highest population of this age class was in December; adults were present throughout the year, but the highest numbers of captures were in April and May (Table 1). Offspring from DA were found from August to November, with peak density in August; juveniles appeared from October to March, with density peaking in December; and adults were seen from September to July, with the greatest densities from April to May (Table 1).

Growth Rate

The length logistic model showed the best fit to the growth rate data for males and females from UA; in contrast, the Von Bertalanffy model showed the best fit to the growth rate data for both males and females of DA (Fig. 2 and Table 2). The logistic model by length showed that growth rates for males ($r \pm EE$: 0.007 ± 0.0005) and females ($r \pm EE$: 0.008 ± 0.0008) from UA were similar, whereas the asymptotic growth curve of females ($A_1 \pm$

Table 1. Numbers of *Sceloporus spinosus* individuals in each age class during each study month at Yagul Natural Protected Area. UA = Undisturbed area, DA = Disturbed area. * Indicates the rainy months. A = adult, J = juvenile.

UA	Offspring		Juveniles		Adults		Recaptures
Samples	Males	Females	Males	Females	Males	Females	
January	0	0	1	2	1	0	0
February	0	0	3	4	2	1	1♀A
March	0	0	2	4	7	2	3♂A, 1♀, 2♀J
April	0	0	3	3	9	9	4♂A, 5♀, 2♂J
May	0	0	0	0	7	10	4♂A, 5♀A
June*	0	0	0	0	2	6	1♂A, 5♀A
July*	0	1	0	0	7	4	5♂A, 2♀A
August*	3	1	0	0	2	3	2♀, 1♂J
September*	3	2	0	0	5	1	3♂A, 1♀A, 2♂J
October	3	1	0	1	4	3	3♂A, 2♀A, 2♂J, 1♀J
November	4	0	3	2	6	0	5♂A, 2♂J, 1♀J
December	1	0	7	2	2	0	5♂A, 3♀A
Density (#/area)	0.0019		0.0037		0.0093		
Total	14	5	19	18	54	39	73

DA	Offspring		Juveniles		Adults		Recaptures
Samples	Males	Females	Males	Females	Males	Females	
January	0	1	2	2	2	2	0
February	0	0	1	0	2	1	0
March	0	0	2	2	5	3	1♂A
April	0	0	0	0	10	6	6♂A, 3♀A
May	0	0	0	0	11	10	4♂A, 5♀A
June*	0	0	0	0	1	5	2♀A, 1♂A
July*	0	0	0	0	4	5	5♂A, 2♀A
August*	3	4	0	0	0	0	0
September*	1	2	0	0	1	3	0
October	1	0	2	4	0	2	1♀J, 2♀A
November	1	0	4	2	0	2	1♀A, 3♂J
December	0	0	5	6	1	1	1♂A, 1♀, 2♀J
Density (#/area)	0.0013		0.0032		0.0077		
Total	6	7	16	16	37	40	40

EE: 105.359 ± 3.225 mm) was slightly higher than that of males ($A_1 \pm$ EE: 101.706 ± 1.587 mm; Table 2). On the other hand, for males and females from DA, the Von Bertalanffy model showed that male ($r \pm$ EE: 0.005 ± 0.0008) and female ($r \pm$ EE: 0.005 ± 0.0011) growth rates were similar (Table 2); however, the females reached an asymptotic size (maximum size) that was slightly larger ($A_1 \pm$ EE: 108.058 ± 4.139 mm) than the males ($A_1 \pm$ EE: 103.253 ± 3.723 mm; Table 2). Average values of the residuals of growth rates for males and females from UA, obtained with both the logistic by length model and a two-way ANOVA, did not show significant differences (males: -0.001 ± 0.009 ; females: 0.003 ± 0.019) between dry ($F_{1,29} = 0.047$, $P = 0.8300$) and wet seasons ($0.001 \pm$

0.010 , -0.004 ± 0.011 , respectively), between sexes ($F_{1,29} = 0.003$, $P = 0.9577$), or interactions between factors (season*sex; $F_{1,29} = 0.116$, $P = 0.7358$). A similar pattern occurred for DA, where no differences were found in males (-0.003 ± 0.013) and females (-0.007 ± 0.015) between dry and wet seasons ($F_{1,24} = 0.975$, $P = 0.3333$; males = 0.021 ± 0.009 and females = 0.015 ± 0.033), between sexes ($F_{1,24} = 0.044$, $P = 0.8358$), or interactions between factors (season*sex; $F_{1,24} = 0.004$, $P = 0.9472$).

On the other hand, there were no differences in the overall growth rates of males from the two populations ($F_{1,31} = 0.234$, $P = 0.6317$), in neither dry (UA: -0.001 ± 0.009 ; DA: -0.003 ± 0.013) nor wet (UA: 0.001 ± 0.010 ; DA: 0.021 ± 0.009) seasons ($F_{1,31} = 0.535$, $P = 0.4699$);

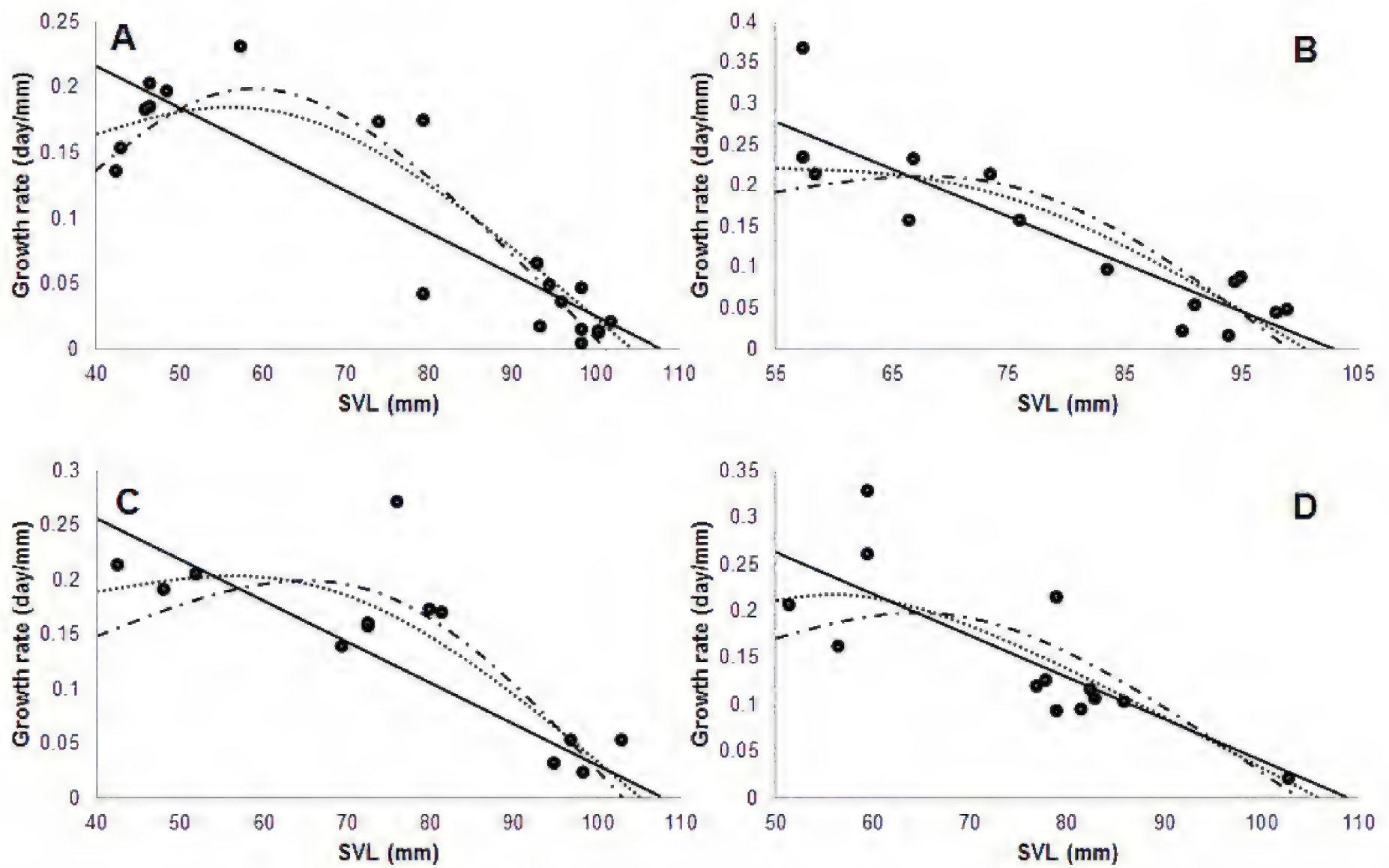


Fig. 2. Growth rate of *Sceloporus spinosus*. (A) Undisturbed area (UA) males, (B) Disturbed area (DA) males, (C) UA females, and (D) DA females. Black circles represent data points for individual lizards. Modeled relationships between growth and body sizes of males and females: solid lines = Von Bertalanffy, dashed lines = logistic by length, and dotted lines = logistic by mass.

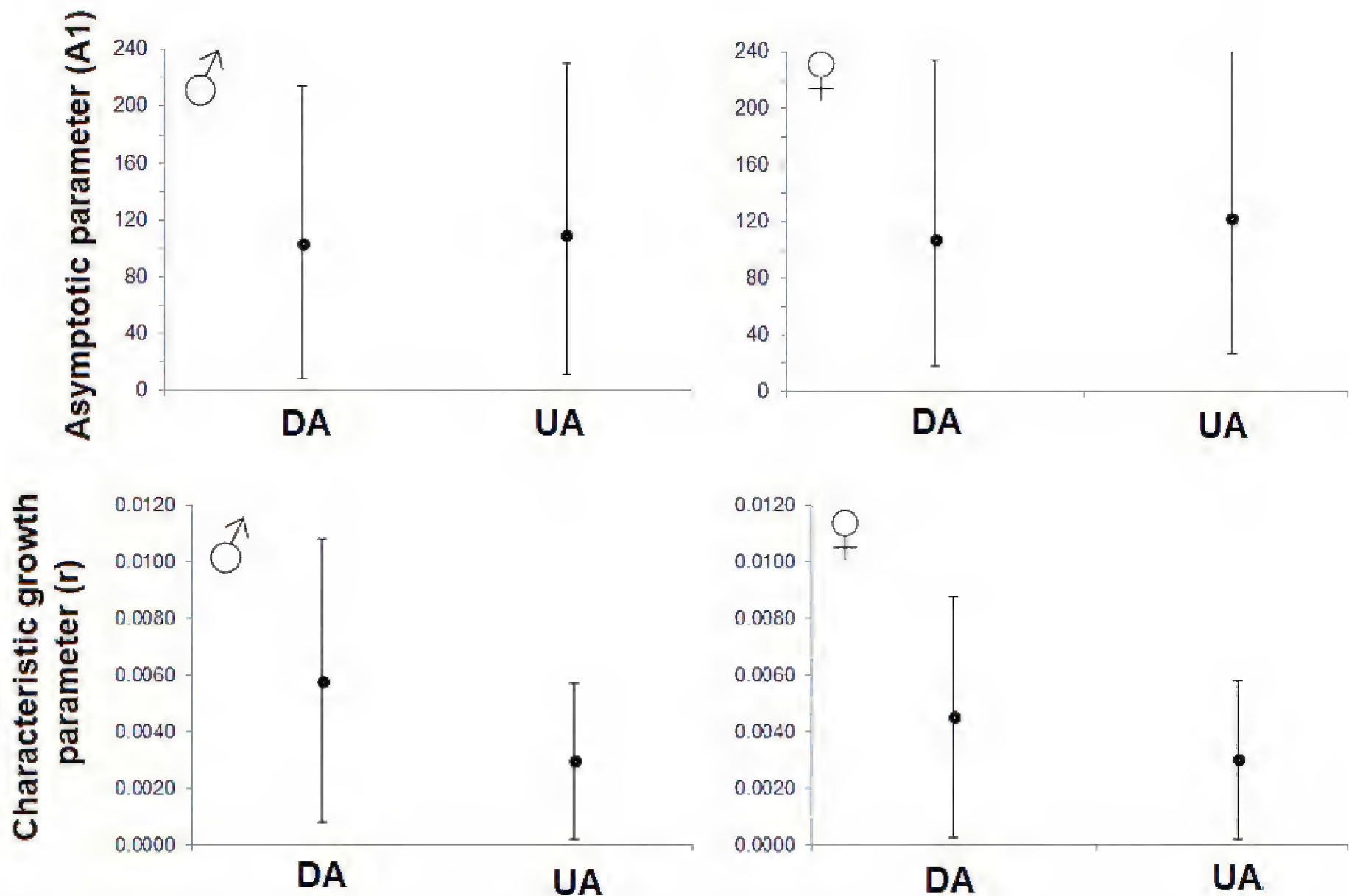


Fig. 3. Means and 95% confidence intervals of the Asymptotic growth (A_1) and Characteristic growth (r) parameters obtained by the Von Bertalanffy and logistic by length models for males and females of *Sceloporus spinosus* in both Disturbed area (DA) and Undisturbed area (UA) populations.

therefore, the interaction term (locality*season) was not significant ($F_{1,31} = 0.401$, $P = 0.5312$). The same pattern was found in females, as there were no differences between localities ($F_{1,22} = 0.057$, $P = 0.8130$), or seasons ($F_{1,22} = 0.124$, $P = 0.7284$); and, therefore, the interaction term (locality*season) was also not significant ($F_{1,22} = 0.587$, $P = 0.4519$).

Growth parameters (r and A_1) were similar for males and females in UA and DA populations (Fig. 3); confirming that there is no difference between the sexes. In UA, the growth rate showed that males reached sexual maturity at SVL 85 mm at age 210 d (7 months), whereas females attained sexual maturity at SVL 89 mm at age 280 d (9 months). In DA, males reached sexual maturity at SVL 81 mm at age 210 d, and females at SVL 85 mm at age 280 d.

Survival Model Estimation

To analyze survival rate (ϕ) and recapture (p) of *S. spinosus* in UA and DA populations, a set of models was developed (Table 3). The single model that described survival rate as varying between groups (sexes) and where the recapture rate was constant [$\Phi(\text{sex})p(c)$] was chosen as the best fit for both populations (Table 3A). Based on this model, the survival rate for males from

UA (0.82) was higher than that of females (0.70), while the recapture rate was similar for both sexes (0.40; Table 4A). In addition, the survival rate for males from DA (0.75) was higher than that of females (0.65), and the probability of recapture was higher for males (0.40) than for females (0.35; Table 4A). These values are lower than those found for UA, which suggests a higher probability of survival and recapture in UA than DA. On the other hand, survival and recapture rates by season (wet and dry) in UA and DA populations showed that the model with the best fit was $\Phi(c)p(\text{season})$, indicating that survival rate is constant, and the probability of recapture varies between seasons (Table 3B). According to this model, the survival rates in both populations were higher in the dry (UA: 0.76, DA: 0.92) than the wet (UA: 0.54, DA: 0.49; Table 4B) season.

Discussion

More lizards were marked at the UA site than the DA site during this study. However, at both UA and DA the greatest numbers of adult recaptures were in April and May, due to the peak in reproductive activity during these months (Valdéz-González and Ramírez-Bautista 2002). According to the recapture data, lizards born in August-September reached the minimum SVL at sexual

Table 2. Growth parameters for *Sceloporus spinosus* males and females from UA and DA populations obtained from each growth model. RMS = residual mean square, R^2 = coefficient of determination, A_1 = asymptotic of growth, r = parameter of growth, $\pm$ = standard error.

UA	Model	RMS	R^2	A_1	r
Males ($n = 20$)					
	Von Bertalanffy	0.029	0.730	109.480 $\pm$ 5.223	0.003 $\pm$ 0.0003
	Logistic by length	0.019	0.849	101.706 $\pm$ 1.587	0.007 $\pm$ 0.0005
	Logistic by weight	0.025	0.797	100.081 $\pm$ 1.114	0.012 $\pm$ 0.0009
Females ($n = 13$)					
	Von Bertalanffy	0.029	0.595	122.243 $\pm$ 12.423	0.003 $\pm$ 0.0007
	Logistic by length	0.019	0.736	105.359 $\pm$ 3.225	0.008 $\pm$ 0.0008
	Logistic by weight	0.023	0.687	102.482 $\pm$ 2.221	0.012 $\pm$ 0.0011
DA	Model	RMS	R^2	A_1	r
Males ($n = 15$)					
	Von Bertalanffy	0.027	0.809	103.253 $\pm$ 3.723	0.005 $\pm$ 0.0008
	Logistic by length	0.035	0.756	99.762 $\pm$ 2.410	0.0099 $\pm$ 0.0010
	Logistic by weight	0.048	0.664	98.523 $\pm$ 1.995	0.0141 $\pm$ 0.0016
Females ($n = 13$)					
	Von Bertalanffy	0.030	0.625	108.058 $\pm$ 4.139	0.005 $\pm$ 0.0011
	Logistic by length	0.032	0.601	100.178 $\pm$ 3.225	0.008 $\pm$ 0.0012
	Logistic by weight	0.429	0.046	99.340 $\pm$ 3.7378	0.012 $\pm$ 0.0017

Table 3. Models describing survival rate (ϕ) and recapture (p) of *Sceloporus spinosus* males and females in UA and DA with the Jolly-Saber model using the Mark program. The models are fitted with the Mark program considering that Φ y p (probability of survival and recapture) can be either constant (c) or varying between sex (s), season (dry and wet), and population. AIC = Measurement of the level of adjustment and parsimony of each model, Δ_i = difference of AIC, W_i = weight of AIC, K = number of parameters.

A. Models for estimation of sex and populations.

Model	AIC	Δ_i	W_i	K
Φ (sex) $p(c)$	231.98	0	0.69	12
Φ (c) $p(c)$	234.09	2.11	0.24	13
Φ (population) $p(c)$	238.22	6.25	0.03	12
Φ (population) $p(\text{sex})$	240.54	8.57	0.01	13
Φ (sex) $p(c)$	241.68	9.71	0.01	3
Φ (c) $p(\text{sex})$	242.11	10.13	0	3
Φ (population) $p(\text{population})$	243.39	11.42	0	21
Φ (sex) $p(\text{sex})$	243.84	11.86	0	4

B. Models for estimation of season (dry and wet) and populations.

Model	AIC	Δ_i	W_i	K
Φ (c) $p(\text{season})$	227.02	0	0.88	3
Φ (season) $p(c)$	231.98	4.96	0.07	12
Φ (season) $p(\text{population})$	237.17	10.16	0.01	3
Φ (c) $p(c)$	240.11	13.1	0	2
Φ (t) $p(t)$	243.39	16.38	0	21
Φ (population) $p(\text{season})$	247.87	20.86	0	2

maturity (> 80 mm) in April-May of the following year, a similar pattern seen in another population of the same species (Valdéz-González and Ramírez-Bautista 2002). Recruitment of offspring, together with adult males and females, and a few subadults, results in population growth with respect to these age structures. These events are synchronized with an increase in ambient temperatures (from 20.9 °C in April to 21.2 °C in May) and precipitation (from 113.3 mm in June to 114.4 mm in May) in the region, which also coincide with high production of food in the environment (Dunham 1982; Ramírez-Bautista and Vitt 1997).

In general, these results revealed that lizard density was significantly similar between populations, but not between seasons (higher in the wet season than in the dry season). The higher density of lizards found in the wet season could be explained by a high supply of the food consumed by this species. However, this assumption brings up additional questions regarding the feeding habits of other species living in sympatry with *S. spinosus* in disturbed and undisturbed environments. Therefore, it is necessary to investigate whether those sympatric species have the same possibilities of acquiring available resources (food and microhabitat), or if they

display different activity schedules that allow them to obtain resources more efficiently. Such studies would certainly expand our knowledge on the natural history of this species, and consequently enable the development of more effective conservation strategies.

The growth rate models used here showed that males and females in both populations grow at the same rate and reach maximum SVL at similar sizes. These results were found by the logistic model by length (UA) and Von Bertalanffy (DA), models that are known to fit most growth analyses for at least some lizard species of the family Phrynosomatidae (Lemos-Espinal and Ballinger 1995; Zuñiga-Vega et al. 2008; Pérez-Mendoza et al. 2014; Ramírez-Bautista et al. 2016). The pattern of low growth rate variation in both populations observed in this study could suggest that populations of this species are able to inhabit areas with certain degrees of disturbance, as has been shown in other species of lizards analyzed by D'Cruze and Kumar (2011) in both disturbed and undisturbed environments.

On the other hand, a homologous pattern in growth rates for males and females in both UA and DA populations may be due to the similarities in SVL at birth and the SVL at sexual maturity. For the former (SVL at birth), growth rates are likely to be regulated by predation intensity, acting mainly on offspring and juveniles of both populations (Schoener 1979; Andrews 1982). This interpretation could be different if both demographic parameters (survivorship and recaptures) evaluated for each site had changed based on an increased number of recaptures. This pattern has also been observed in populations of *S. grammicus* from Central Mexico (Pérez-Mendoza et al. 2013, 2014).

The life history characteristics studied here for this species could have significant plasticity among its populations, and therefore, small differences in temperature, precipitation, and food between UA and DA would not have apparent effects (Valdez-González and Ramírez-Bautista 2002; Valencia-Limón et al. 2014). A similar pattern in growth rates also occurs between sexes and age classes in other lizard genera (e.g., *Xenosaurus* spp.; Molina-Zuluaga et al. 2013).

The low variation in SVL at sexual maturity observed in the growth curve within and between populations is partially explained by the absence of sexual dimorphism with respect to SVL (Valdéz-González and Ramírez-Bautista 2002; Ramírez-Bautista et al. 2013). Walkup et al. (2017) pointed out that *Uta stansburiana*, *Aspidoscelis marmorata*, *A. sexlineata*, and *Sceloporus consobrinus* present generalist habits in microhabitat choice, being able to inhabit sites with different degrees of disturbance. Consequently, these species tend to present reduced variation in some of their demographic characteristics, as a measure of phenotypic plasticity towards different degrees of environmental disturbance.

In this study, lizards from UA were numerically more abundant and showed a slightly higher survival rate

Table 4. Probability of total survival (ϕ) and recapture (p) for both sexes and season for UA and DA populations. SE = standard error.**A. Survival values and recapture between sexes**

Undisturbed area (UA)			Disturbed area (DA)		
Parameters	Estimation	SE	Parameters	Estimation	SE
Φ (males)	0.822	0.042	Φ (males)	0.75	0.019
p	0.402	0.051	p	0.40	0.024
Φ (females)	0.706	0.056	Φ (females)	0.65	0.061

B. Survival values and recapture between seasons

Undisturbed area (UA)			Disturbed area (DA)		
Parameters	Estimation	SE	Parameters	Estimation	SE
Φ (dry)	0.765	0.036	Φ (dry)	0.927	0.078
Φ (wet)	0.538	0.074	Φ (wet)	0.489	0.072

than those from DA. This suggests that relative density may be a measure of population variation that predicts the survival value for both sites. However, in some cases lower survival probability has been observed in populations with high density than in populations with low density (Stearns 1992; Zúñiga-Vega et al. 2008).

Survival rates and probabilities of recapture obtained here for males and females were higher compared to those reported for *Anolis nebulosus* (Hernández-Salinas 2014), *Xenosaurus grandis* (Zúñiga-Vega et al. 2007), and *Sceloporus grammicus* (Pérez-Mendoza et al. 2014). The models used in these studies considered sex, season (dry and wet), and populations as variables that express the greatest sources of variation, similar to the survival rate assessed in other species of the genus *Sceloporus* (Zúñiga-Vega et al. 2008; Pérez-Mendoza et al. 2014). The results for survival obtained here were similar to those of other studies (Zúñiga-Vega et al. 2008; Molina-Zuluaga et al. 2013; Hernández-Salinas 2014) where survival and recapture rates were found to be higher in males and females in one population during the dry season. One possible explanation is that the reproductive activity of this species begins during the rainy season, causing males to search for mates for reproduction, participating in male-male agonistic competition, and consequently becoming more susceptible to predation (Sinervo et al. 1991). In contrast, for females, survival rates have been observed to decrease dramatically at the end of the gestation stage and parturition (end of the rainy season), possibly because pregnant females become heavier and slower, therefore increasing the risk of predation during the wet season (Clobert et al. 1998, 2000; Stearns 1992; Zúñiga-Vega et al. 2008). This pattern also has been observed in other lizard species, such as *Anolis nebulosus* from tropical dry forest (Ramírez-Bautista and Vitt 1997).

Conclusions

This study found little variation regarding growth rate, survival, and body size at sexual maturity between

populations of *Sceloporus spinosus*. This supports the assumption that the toe-clipping method did not impair the performance of individuals in both populations, allowing the results of this study to be comparable with future works. For the above, and contrary to our expectations, this study showed that males and females in both UA and DA showed similar growth rate patterns, with both sexes reaching sexual maturity at similar SVL in both populations. The results obtained here may not be regarded as the variation typically observed in these life history attributes (growth, survival, and SVL at maturity), since the minimal variation observed in these characters may indicate that they are genetically fixed components, as has been determined in some species of *Xenosaurus* (Zúñiga-Vega et al. 2005, 2007; Zúñiga-Vega 2011) and *A. nebulosus* (Ramírez-Bautista and Vitt 1997). The results of this study should be taken with caution, since further studies are needed to determine the effects of temperature, precipitation, competition, and food on the life-history characteristics of the species living in both environments. Additional studies will support more solid conclusions regarding the growth, survival rate, and size at sexual maturity across the entire distribution range. In order to improve these results, we also suggest the development of hypotheses aimed at determining the relative abundance of predators. Furthermore, it is necessary to develop elasticity and sensitivity analyses with larger sets of capture-recapture data to better comprehend the effects of environmental pressures on the life history characteristics of a species that is found in both intact environments and environments with varying degrees of disturbance.

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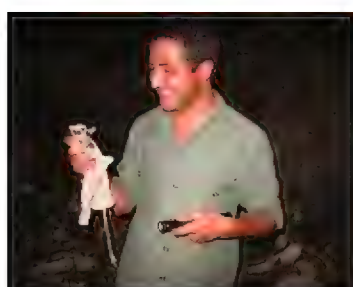
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Carlos A. Torres Barragán is a biologist and herpetologist born in Oaxaca, Mexico. Carlos graduated from Universidad Autónoma “Benito Juárez” de Oaxaca (UABJO) in Mexico, where he worked on the ecology of the amphibian and reptile communities of Yagul Natural Protected Area in Oaxaca. He later earned his Master’s degree at Instituto Politécnico Nacional (IPN-CIIDIR-Oaxaca) in 2015, where he studied demographic parameters of reptiles living in disturbed and undisturbed sites within Yagul Natural Protected Area. Currently, Carlos works as a consultant with indigenous communities in different regions of Oaxaca, in areas regarding biodiversity monitoring, sustainable and friendly-biodiversity productive systems, and development of local technical capabilities.



Uriel Hernández Salinas is a Mexican herpetologist interested in the richness, diversity, biogeography, and conservation of amphibians and reptiles in central and northern Mexico. Uriel is a full-time professor at CIIDIR Durango of the Instituto Politécnico Nacional and curator of the scientific collection of amphibians and reptiles at that research institution.



Aurelio Ramírez Bautista is a full-time professor at Universidad Autónoma del Estado de Hidalgo. Currently, Aurelio teaches undergraduate and graduate biology courses, including those on population ecology, herpetology, life history evolution, and the reproductive biology of amphibians and reptiles. His research focuses on the study of biodiversity (species richness), biogeography, and conservation of amphibians and reptiles of Mexico, as well as life history evolution in lizard species from the temperate, tropical, and desert environments of Mexico.



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A new gecko genus from Zagros Mountains, Iran

^{1,2,3}Farhang Torki

¹Razi Drug Research Center, Iran University of Medical Sciences, Tehran, IRAN ²FTEHCR (Farhang Torki Ecology and Herpetology Center for Research), 68319-16589, P.O. Box: 68315-139 Nourabad City, Lorestan Province, IRAN ³Biometrical Center for Researches (BMCR), Khalifa, Nourabad, Lorestan, IRAN

Abstract.—A new genus and species of gekkonid lizard is described from the Zagros Mountains, western Iran. The genus *Lakigecko* gen.n. can be distinguished from other genera of the Middle East by the combination of the following characters: depressed tail, strongly flattened head and body, eye ellipsoid (more horizontal), and approximately circular whorls of tubercles (strongly spinose and keeled).

Keywords. Gekkonidae, *Lakigecko* gen.n., *Lakigecko aaronbaueri* sp.n., new species, Reptilia, Sauria, Western Iran

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Introduction

Thus far, 49 species and 14 genera of Gekkonidae have been recorded in Iran (Safaei-Mahroo et al. 2016). Ten new species of gekkonid lizards have been described since 2000, including seven new species and a recently described monotypic gecko genus, *Parsigecko*, from southern Iran (Safaei-Mahroo et al. 2016). This study adds another new gecko genus to the fauna of Iran based on the species described herein. In addition, this study includes a discussion of the validity of genus *Carinatogecko* Golubev and Szczerbak, 1981.

Materials and Methods

During field trips carried out on the Iranian plateau, the new taxon was collected from Nourabad, Lorestan Province, Zagros Mountain, western Iran (Fig. 1). The holotype and paratype specimens of this new species have been deposited in the MTD (Senckenberg Naturhistorische Sammlungen, Museum für Tierkunde, Dresden, Germany).

Measurements. The following measurements were taken with a digital caliper, and interpolated to the nearest 0.1 mm: SVL: snout-vent length (from tip of snout to anterior margin of cloaca); TaL: tail length (from posterior margin of cloaca to tip of tail, original or regenerated); TaL/TL: ratio tail length/total length; TW: tail width (taken at base of tail); TH: tail height (taken at base of tail); TW/TH: ratio tail width/tail height; HL:

head length (from tip of snout to posterior margin of ear); HH: maximum head height (from occiput to undersides of jaws); HW: maximum head width; HL/SVL: ratio head length/snout-vent length; EDH: eye diameter horizontal; EDV: eye diameter vertical; IO1: anterior interorbital distance; IO2: posterior interorbital distance; EE: distance between posterior margin of eye to posterior margin of ear opening; EaD: ear diameter vertically; IN: internostril distance; IL: interlimbs; FL: forelimb length; and HL: hindlimb length.

Lepidosis. The following scale counts were taken, and on the right side of body where appropriate: N: nasals (nasorostrals, supranasals, postnasals, first supralabial); ISN: intersupranasals (scales between supranasals, in contact with rostral); SPL: supralabials; IFL: infralabials; IO: interorbitals (number of scales in a line between anterior corners of eyes); PO: preorbitals (number of scales in a line from nostril to anterior corner of the eye); PM: postmentals; LT: number of subdigital lamellae (on toes counted from the first broad lamella to the claw); DC: number of dorsal crossbars; and TC: number of tail crossbars.

Comparisons. For comparison, representatives of five other naked-toed gekkonid genera were examined and compared with the new genus as follows: *Cyrtopodion* (*C. scabrum*: $n = 9$, FTHM 004600–08; *C. kiabii*: $n = 8$, FTHM 004880–87); *Temnidactylus* (*T. caspius*: $n = 11$, FTHM 004851–61); *Mediodactylus* (*M. russowii*, $n = 2$; *M. heterocercus*, $n = 5$; *M. sagittifer*, $n = 3$; *M.*

Correspondence. torkifarhang@yahoo.com



Fig. 1. Map showing the type locality of *Lakigecko aaronbaueri* sp.n. in the Garmabe region, Nourabad, Lorestan Province, Iran.

spinicauda, $n = 3$), *Carinatogekko* (*C. heteropholis*: $n = 29$; FTHM 003400–003428; *C. aspratilis*, $n = 12$, FTHM 003300–003311; *C. ilamensis*, $n = 3$), *Bunopus* (*B. tuberculatus*: $n = 12$, FTHM 003200–11). Other data were taken from Minton et al. (1970), Anderson (1973, 1999), Golubev and Szczerbak (1981), Nazarov et al. (2009), Ahmadzadeh et al. (2011), Fathinia et al. (2011), Torki (2011), and Safaei-Mahroo et al. (2016). Abbreviations: TT: Tail tubercle distribution (D: dorsal; L: dorsolaterals; V: ventral); TS: tail shape (S: squat, i.e., short and thick; Q: not squat, i.e., more elongate); LT: Lamella type (M: smooth; K: keeled); DT: dorsal tubercles in each segment (C: in contact; D: not in contact); DS: dorsal scales between whorls (E: elevated; N: not elevated); GS: digits shape (A: angular; S: sub-angular; W: weakly angular; N: not angular); FP: femoral pores (+: present; -: absent); ES: eye shape (G: Globular; E: Ellipsoid); and HA: head angle (degree of lateral view between lower ear-nostril-upper eye).

Systematics

Lakigecko gen.n.

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Type species: *Lakigecko aaronbaueri* sp.n., herein described.

Definition: The monotypic genus *Lakigecko* gen.n. differs from all other genera of the Family Gekkonidae by the following combination of characters: (1) depressed tail; (2) much flattened head and body; (3) acute head angle (laterally); (4) eye horizontally ellipsoid (horizontal/vertical ratio in life more than 1.2 and preserved approximately 1.6); (5) whorls of tubercles

Table 1. Morphometric measurements (in mm) and scalation of *Lakigecko aaronbaueri* sp.n., abbreviations as defined in the text. * end of tail regenerated; ** most part of tail regenerated.

Characters	Holotype (MTD 49500)	Paratype (MTD 49499)
SVL	32.79	32.19
TaL	25.58*	25.75**
TaL/TL	0.78	0.80
TW	3.06	3.24
TH	2.48	1.95
TW/TH	1.23	1.66
HL	8.04	8.10
HH	3.48	3.40
HW	6.45	6.27
HL/SVL	0.24	0.25
EDH	2.25	2.30
EDV	1.21	1.59
IO1	3.27	3.23
IO2	4.38	4.58
EE	2.68	2.78
EaD	0.70	0.63
IN	0.98	1.02
IL	14.80	13.32
FLL	11.81	11.26
HLL	16.90	15.91
N	5	5
ISN	1	1
SPL	9	10
IFL	7	7
IOS	13	12
PO	9	10
PM	3	3
L4T	17	19

approximately circular (extended onto ventrolateral surface): strongly spinose and keeled caudal tubercles that extend onto dorsolateral and ventrolateral surfaces, lateral tubercles larger than mid-dorsal and ventral, mid-dorsal and ventral tubercles are clearly different from smaller subcaudal scales, whorls separated by two rows of keeled scales; (6) spinose tubercles extend along entire length of tail (tubercle shape consistent throughout); (7) mid-dorsal tubercle rows separated from each other by two scales, dorsolateral rows of tubercles (four tubercle rows on each side) are in contact with one another; (8) dorsolateral scales strongly elevated, pyramidal, and keeled; (9) dorsal scales mostly smooth; and (10) without femoral or precloacal pores; two postcloacal pores (openings to the postcloacal sacs).

Distribution: Known only from the type locality.

Etymology: The generic nomen *Lakigecko* is derived from the word “Laki” which refers to the Lak region near the type locality.

Comparisons (Fig. 2, Table 2): Differs from *Cyrtopodion* Fitzinger, 1843 by having small, spiny, and strongly keeled subcaudal scales (vs. smooth, plate-

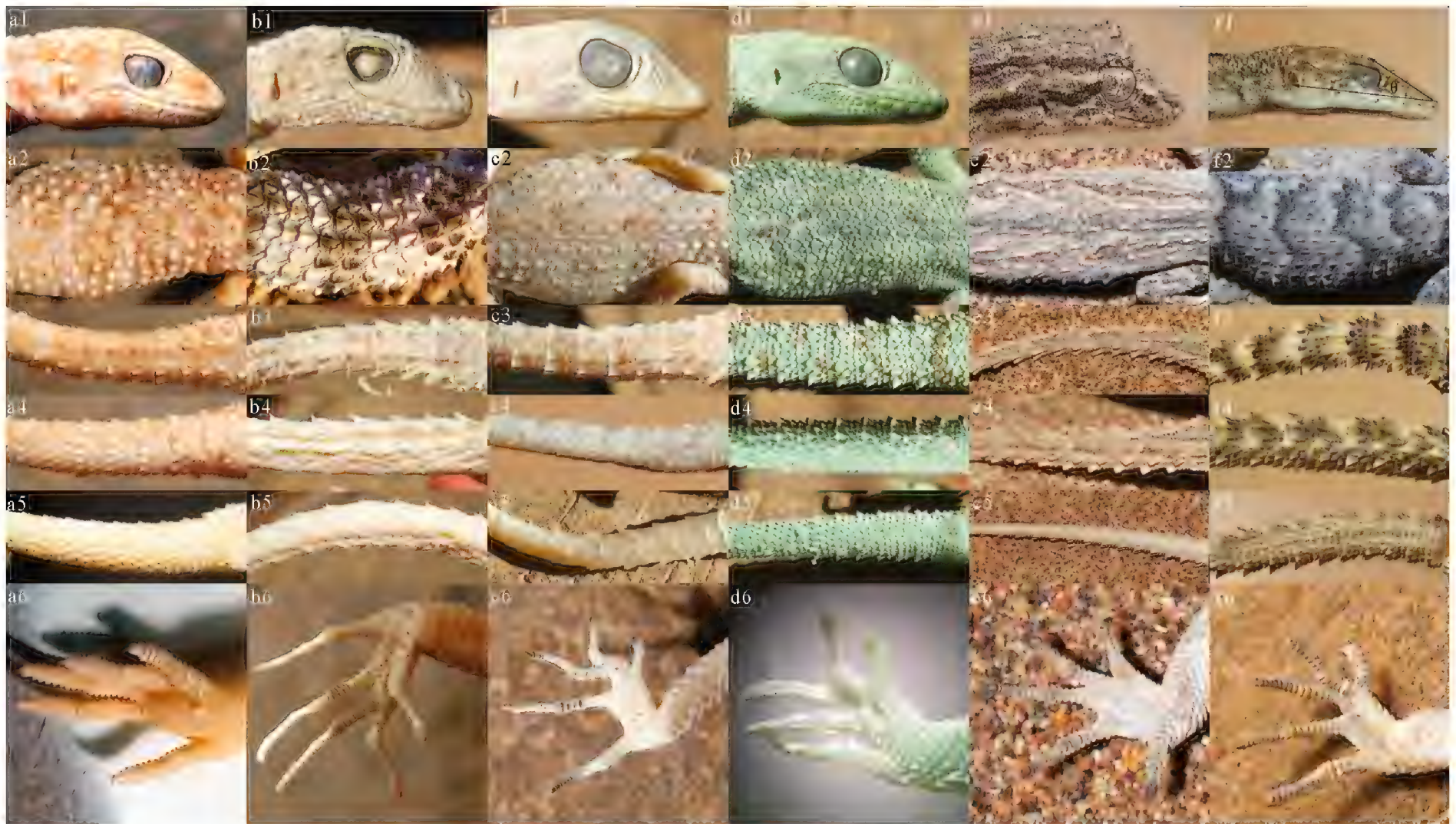


Fig. 2. Comparisons of (1) head shape, (2) dorsal view, (3) dorsal view of tail, (4) lateral view of tail, (5) ventral view of tail, and (6) subdigital lamella of hindlimbs, in the new gecko with other genera. (a) *Bunopus tuberculatus*, (b) *Temuidactylus caspius*, (c) *Cyrtopodion scabrum*, (d) *Carinatogekko heteropholis*, (e) *Mediodactylus russowii*, and (f) *Lakigecko aaronbaueri* sp.n. Photos by F. Torki (a–d, f) and B. Safaei-Mahroo (e).

like subcaudals); dorsal scales elevated and overlapping (vs. non-elevated or flattened cobble-stone shaped) and a greatly flattened and elongate head (vs. head oval and more massive); from *Mediodactylus* Szczerbak and Golubev, 1977 by having keeled, tubercular scales forming the end of each caudal segment (vs. in the middle of each segment), at least 14 sharply keeled tubercles in each caudal whorl (vs. a semicircle of six whorls: three on left and three on right), caudal tubercles in contact with one another laterally (vs. not in contact), caudal tubercles forming a relatively complete ring around the tail (vs. distributed only on the dorsal half of the tail), and a flattened and elongate head (vs. not); from *Carinatogekko* Golubev and Szczerbak, 1981 by smooth subdigital lamellae on the forelimbs and hindlimbs (vs. keeled transverse subdigital lamellae), and smooth dorsal scales (vs. strongly keeled); from *Temuidactylus* Szczerbak and Golubev, 1984 by the lack of femoral pores (vs. more than 20 femoral pores), ventral subcaudals small and strongly keeled (vs. plate-like and smooth), 14 sharply keeled tubercles in each tail whorl segment (vs. six tubercles per whorl); and a flattened and elongated head

(vs. not). In general, the new genus is easily differentiated from *Cyrtopodion*, *Temuidactylus*, *Mediodactylus*, and *Carinatogekko* by its acute head angle (less than 30° vs. 40°, 40°, 35°, and 35°, respectively), strongly flattened head, depressed tail, more caudal tubercles, spinose caudal tubercles, and elliptical eye shape.

Lakigecko gen.n. can be distinguished from *Agamura* Blanford, 1874 by its large and strongly keeled caudal tubercles (vs. not), limbs robust (vs. slender), enlarged postmentals (vs. not enlarged), and tail broad (vs. slender); from *Bunopus* Blanford, 1874, by subdigital lamellae completely smooth (vs. subdigital lamellae with free margin under magnification); 14 sharply keeled tubercles in each whorl segment of tail (vs. 4–6 and not sharp and spiny); from *Rhinogekko* de Witte, 1973 by nasal scales not forming a cylindrical caruncle (vs. nostril at apex of prominent swollen or cylindrical caruncle formed by the nasal scales); from *Microgekko* and *Tropicolotes* Peters, 1880 by having uniform dorsal scales, large scales, and keeled tubercles on the dorsum of body and tail (vs. dorsal scales heterogeneous, scales small, and without keeled

Table 2. Comparisons of *Lakigecko* gen.n. with other related genera. For abbreviations see *Materials and Methods* section.

Characters	TT			TS	LT	DT	DS	GS	FP	ES	HA
	D	L	V								
<i>Cyrtopodion</i>	+	+-	-	B	M	C	N	A	-	G	≥40°
<i>Temuidactylus</i>	+	+-	-	B	M	C	N	A	+	G	≥40°
<i>Bunopus</i>	+	+	-	B	K	C	N	N	-	G	≥40°
<i>Mediodactylus</i>	+	+	-	B	M	D	N	S	-	G	≥35°
<i>Carinatogekko</i>	+	+-	-	B	K	D	E	W	-	G	≥35°
<i>Lakigecko</i> gen.n.	+	+	+	S	M	C	E	S	-	E	<30°

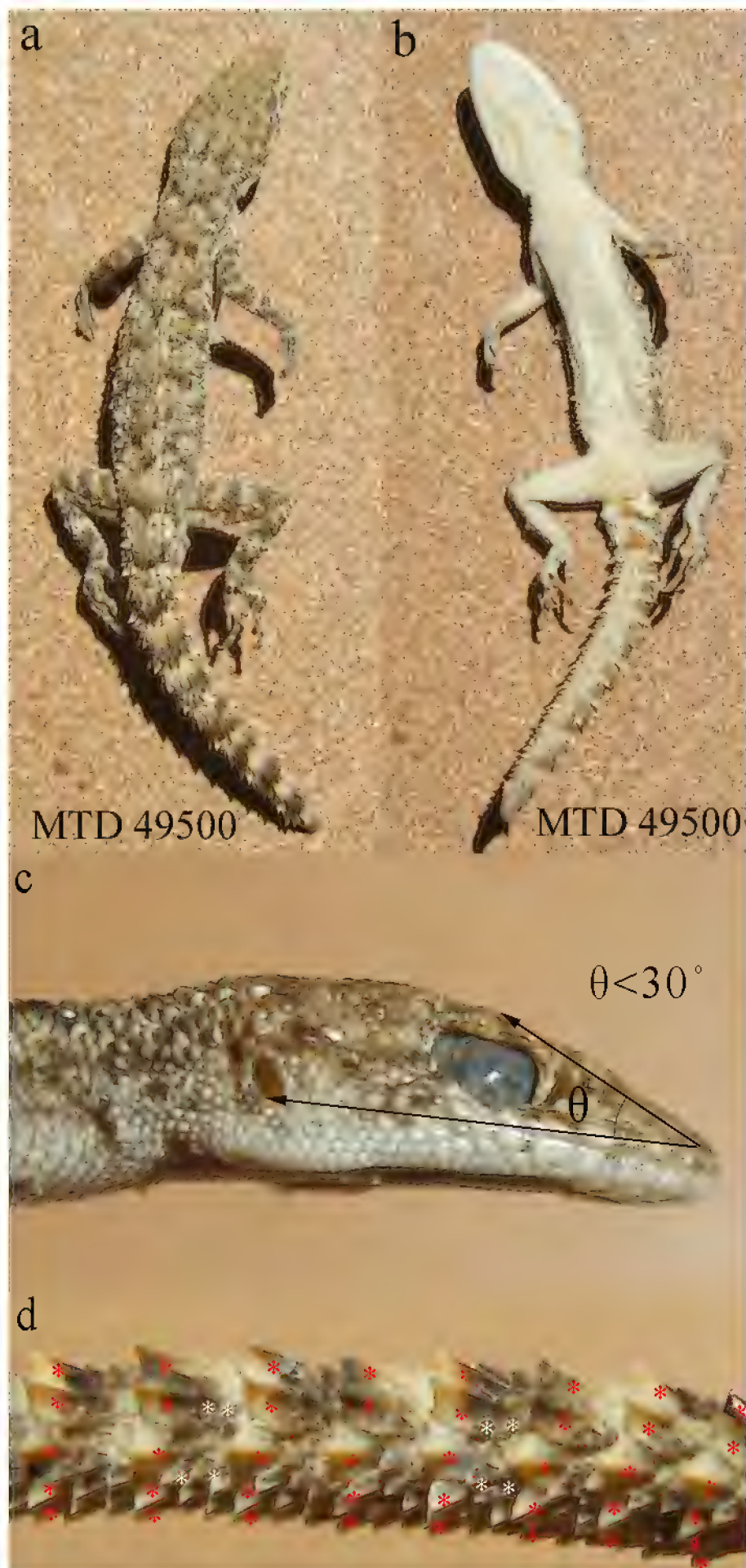


Fig. 3. (a) Dorsal, (b) ventral, (c) lateral head, and (d) lateral tail views of the holotype of *Lakigecko aaronbaueri* sp.n. Red stars: tubercular whorls; white star: keeled scale interspaces.

tubercles on dorsum and tail); from *Hemidactylus* by non-dilated digits (vs. well-defined dilated digital bases); from *Pseudoceramodactylus* Haas, 1957, *Crossobamon* Boettger, 1888, and *Stenodactylus* Fitzinger, 1826 and *Teratoscincus* by digits without elongate fringes (vs. digits with well-defined lateral elongate fringes); from *Parsigecko* Safaei-Mahroo, Ghaffari, and Anderson, 2016 by large, keeled tubercles on body dorsum, subcaudal scales small and strongly keeled (vs. without dorsal tubercles, and subcaudal scales large and smooth).

Contents: At present the new genus includes a single species, *Lakigecko aaronbaueri* sp.n.

Lakigecko aaronbaueri sp.n. (Fig. 3–4)



Fig. 4. Dorsal view in life of (a) holotype and (b) paratype of *Lakigecko aaronbaueri* sp.n. before preservation.

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Holotype (Fig. 3): MTD 49500, adult male, collected on the western slope of the central Zagros Mountains, Garmabe Region, Nourabad, Lorestan Province, western Iran on 8 April 2016 at night (34°03'N, 47°28'E, elevation 1,470 m asl) by Farhang Torki.

Paratype (Fig. 4): Adult male, MTD 49499, same data as for holotype.

Description of holotype: Body flattened; head flattened; tail depressed and strongly covered by sharp tubercles, eye opening clear, pupil vertical.

Dorsal body. Nostril surrounded by five scales (rostral, nasal, and three behind); nasal nearly completely divided; large dorsal tubercles strongly keeled, each dorsal tubercle surrounded by 10 (or 11) scales, 13 dorsal tubercle rows on dorsal, mid-dorsal tubercles separated from each other by one or two scales, dorsolateral tubercles (four on each side of vertebral midline) are in contact with one another, eight rows (4+4: left + right) extend onto tail, dorsolateral tubercles mostly in contact with one another, separated by two small scales in the dorsal midline; most dorsal scales small and smooth, a few scales large and keeled, mid-dorsal scales elevated, dorsolateral scales strongly elevated, keeled and pyramidal in form; keeled dorsal



Fig. 5. Habitat of *Lakigecko aaronbaueri* sp.n. at the type locality in the Garmabe region, Nourabad, Lorestan Province, Iran.

tubercles extend onto neck and occipital region; neck tubercles pyramidal in form, but dorsal tubercles less so; middorsal neck scales swollen (similar to granules) and not overlapping or elevated, dorsolateral neck scales strongly swollen and keeled (pyramidal) and extending in front of and behind ear; neck scales are different in shape compared to dorsum; upper head tubercles (keeled) smaller than dorsum; nearly all head scales granule-like, keeled and heterogeneous (small to large, cycloid to polygonal); interorbital scales keeled and heterogeneous in size and shape; snout scales keeled; arm covered by strongly keeled large scales (same size as dorsal tubercles); elbow scales keeled; tubercles and scales on forearm strongly keeled; digits covered by strongly keeled scales; leg covered by large, strongly keeled, and overlapping scales (similar size to dorsal tubercles), keeled scales only distributed near knee, lateral surface of leg covered by small scales; foreleg with strongly keeled tubercles distributed among keeled scales, back of hand and digits with strongly keeled scales.

Ventral body. Mental pentagonal; supralabials nine; infralabials seven; three postmentals (PM), 1st PMs in contact with one another, 2nd PMs separated by two scales, 3rd PMs separated by six scales, PMs surrounded by 12 scales, 1st PM in contact with 1st infralabials, 2nd PMs

in contact with 2nd infralabials, and 3rd PMs in contact with 2nd and 3rd infralabials; chin scales small, round, and smooth; scales on neck and between forelimbs smooth and overlapping; interlimb scales keeled, and less overlapped, scale rugosity increases from proximal (across forelimbs) to distal (across hindlimbs) of interlimbs; scales between hindlimb bases as well as preanal scales large and smooth (not keeled); arm scales keeled, elevated; forearm scales keeled and mostly trihedral; forearm scales larger and more rugose than upper arm; scales on palm granular; lamella swollen; dorsolateral scales on digits sharp; thigh as well as foreleg scales keeled and elevated, foreleg scales more rugose than those on thigh; small scales on lateral surface of leg; scales on soles granule-like with some very similar to lamellae, distal lamella smaller; lamellae swollen; dorsolateral scales on digits sharp, lamellae on the middle of each finger (at joint) larger than others; number of lamellae under toes 10:14:15:17:16.

Tail. Mostly original, end of tail regenerated; eight rows of dorsal tubercles extended onto tail; tail tubercles strongly keeled, elevated, sharply keeled, and in contact with each other within each whorl; each whorl formed by 18 (2+8+6+2) tubercles as follows: two in the middle and different from pyramidal scales, eight (4+4) large and elevated and sharp tubercles on dorsum

and extend ventrolaterally, six small (3+3) ventrolateral tubercles distinguishable from subcaudal scales, and finally two small scales that are similar to subcaudal scales; 14 whorls of tubercles are clear on tail, each separated from one another dorsally by two small scales and ventrally by two keeled scales; two rows of small and strongly keeled scales between each whorl (from 1st to 12th whorls), three such rows of scales between 13th and 14th circles.

Color pattern. Background of dorsal body brown, with dark bars covering dorsum and tail; dorsal bars irregular and reticulating; nine dark bars on tail, interspaces yellowish; ventral dirty white; middle of subcaudals bright grayish, lateral surfaces dark grayish; four irregular longitudinal bars on occipital region; dorsum of head covered by small spots; width of bars (wider than interspaces) covering forelimbs (seven bars) and hindlimbs (seven bars); limb bars extend onto digits, five bars on longer digits and four bars on others; dorsal scales on body, tail, limbs, and head are pigmented; ventral body scales pigmented, pigmentation more pronounced laterally than midventrally, two rows of small precloacal scales exhibit the maximum pigmentation, large precloacal scales have minimum pigmentation; scales on venter of leg and foreleg pigmented; all lamellae of pes are dark brownish, middle lamellae less dark; lamellae on digits of manus are light brown, scales on palm are brownish or ashy; chin scales less pigmented; mental, labials, and postmentals pigmented. Color pattern in preservative is similar to life, but paler.

Description of Paratype

Most characters are similar to holotype, some differences are shown in Table 1 and others are: 13 dorsal tubercle rows, four lateral tubercles are in contact with each other and middle tubercles are separated by small scales (same as holotype); tail regenerated, all dorsal and ventral scales are small, strongly keeled, and similar to one another, dorsal scales regular and ventral scales less regular, strongly elevated and overlapping; number of lamellae under toes 11:13:18:19:15.

Color pattern. Five dark irregular bars between limbs; one V-shaped bar on neck; one arcuate bar on occipital; head covered by small spots; all dorsal body bars and spots are blackish; background of dorsal body grayish; tubercles on bars are black and most of those in interspaces are brownish (some of them are both black and brownish in color); regular and irregular bars covering limbs and digits; chin white; ventrals of body and limbs are dirty white; irregular darkish stripes cover dorsum of regenerated tail, all dorsal and ventral scales pigmented; color pattern in preservative is similar to life, but colors are more pale.

Etymology. The species name “*aaronbaueri*” refers to Professor Aaron M. Bauer (Villanova University, Villanova, Pennsylvania, USA) for his major contributions to works on the systematics and morphology of the geckos of the world.

Habitats and ecology. The specimens of *L. aaronbaueri* **sp.n.** were found between stones in the Garmabe region (Nourabad, Lorestan Province, Iran). The type locality is characterized by oak forested hills surrounded by mountains, and is adjacent to the Giz-e-roo River (Fig. 5). Specimens were obtained from the top of the mountains. The new species is syntopic with three reptiles, *Laudakia nupta*, *Ophisops elegans*, and *Heremites aurata*.

Distribution. *Lakigecko aaronbaueri* **sp.n.** is at present known only from the type locality.

Discussion

Previously Červenka et al. (2010) suggested that *Carinatogekko* belonged in the synonymy of *Mediodactylus*. Based on my collections from the Shorab region near Khorramabad (the locality of *Carinatogekko* cf. *heteropholis*, REPT/IRA/1139), Červenka et al. (2010) worked on *Mediodactylus* sp. from Lorestan Province, not *Carinatogekko*. This mistake occurred due to an erroneous taxonomic diagnosis based on two criteria. First, the dorsal scalation type and shape (Červenka et al. 2010; Torki 2011) of *Carinatogekko* cf. *heteropholis* (REPT/IRA/1139) does not support this species belonging to *Carinatogekko*, as the author has previously discussed (Torki 2011). Second, the subdigital lamellae of the new collection do not match the condition of *Carinatogekko* (smooth vs. keeled). Therefore, I suggest that *Carinatogekko* is valid and distinct from the other genera of the *Mediodactylus-Cyrtopodion-Tenuidactylus* group as well as from *Lakigecko* **gen.n.** The number of lamellae, a character cited by Červenka et al. (2010) to confirm that their specimens belonged to *Mediodactylus*, is not an important taxonomic character for the distinguishing of *Carinatogekko* from *Mediodactylus*. Also, the typical cryptic color patterns on the dorsum and tail in *Mediodactylus* are not M- or V-shaped dark transverse bars, as reported by Červenka et al. (2010). Based on my personal observations, there is more variability in the dorsal color patterns in *Mediodactylus* sp. and *Carinatogekko* sp., such as zigzag forms and completely irregular dorsal patterns (this variability is clear in urban house geckos). On the other hand, based on my observations, *Mediodactylus* sp. are distributed mostly in urban houses in Iran, as a result of human activity, rather than in the surrounding habitats. This is also true for some other geckos, such as *Cyrtopodion* and *Hemidactylus*, and for some hair-like pad geckos (Torki 2007; Torki et al. 2008). *Carinatogekko* may

be defined on the basis of at least three important taxonomic characters: (1) all body scales keeled (except intermaxillaries, nasals, chin shields, and labials), (2) subdigital lamellae keeled (not smooth), and (3) subcaudal scales small and strongly keeled. In any case, gecko taxonomy in the Zagros Mountains is a complex problem, largely due to incomplete and poor taxonomic studies to date.

Tail tubercles in *Cyrtopodion*, *Tenuidactylus*, *Bunopus*, and *Lakigecko* **gen.n.** are in contact with one another within whorls, and this is in contrast to *Mediodactylus* and *Carinatogecko* (where they are separated). This character is inconsistent with a recent phylogenetic study (Bauer et al. 2013) that includes some of these taxa. *Tenuidactylus*, *Bunopus*, and *Cyrtopodion* are more closely related to each other and collectively have a sister-taxon relationship with *Mediodactylus*. Both the highly keeled and ventrally

extending tubercles in *Lakigecko* **gen.n.** and the shape of the tail differ from other genera (*Tenuidactylus*, *Bunopus*, *Cyrtopodion*, *Mediodactylus*, *Carinatogecko*). Additionally, the eye shape (horizontally elliptical) and head shape (greatly flattened) of *Lakigecko* **gen.n.** are different from those in these genera. Although, I have not studied the skull anatomy of *Lakigecko* **gen.n.** directly, the external morphology of the head suggests a distinctive skull anatomy of the new genus as well.

In general, four characters of *Lakigecko* **gen.n.** are completely different from related genera (*Tenuidactylus*, *Bunopus*, *Cyrtopodion*, *Mediodactylus*, and *Carinatogecko*) including (Table 2): (1) tail tuberculation, (2) tail shape, (3) eye shape, and (4) head shape. On this basis I suggest that *Lakigecko* **gen.n.** is likely sister to the group *Tenuidactylus-Bunopus-Cyrtopodion* + *Mediodactylus-Carinatogecko*.

Key to the genera of Gekkonidae in Iran (after Anderson 1999; Safaei-Mahroo et al. 2016)

- 1a: Digits strongly dilated.....*Hemidactylus* Oken, 1817
- 1b: Digits not dilated.....2

- 2a: Digits with well-defined lateral fringe of elongate scales.....3
- 2b: Digits without fringe of elongate scales.....5

- 3a: Small dorsal scales intermixed with rounded tubercles.....*Crossobamon* Boettger, 1888
- 3b: Dorsal scales uniform, not intermixed with tubercles.....4

- 4a: Enlarged postmental scales present.....*Pseudoceramodactylus* Haas, 1957
- 4b: No enlarged postmentals.....*Stenodactylus* Fitzinger, 1826

- 5a: Dorsal scales heterogeneous.....6
- 5b: Dorsal scales small (granule like), homogeneous.....13

- 6a: Nostril at apex of prominent swollen or cylindrical caruncle formed by nasal scales, rostral excluded from border of nostril.....*Rhinogecko* de Witte, 1973
- 6b: Nasal scales do not form cylindrical caruncle, although they may appear to be swollen around the nostril; rostral normally forms part of border of nostril.....7

- 7a: Tail cylindrical, very slender, and of almost uniform diameter from base to tip (tip blunt), no mucronate tubercles or annuli.....*Agamura* Blanford, 1874
- 7b: Tail different from above.....8

- 8b: Tail depressed, tail tubercles strongly keeled, spinose, in contact together in at least 14 rows in each whorl, whorl tubercles extended in laterals and ventral of tail; head angle (laterally) less than 30°..... *Lakigecko* **gen.n.**
- 8b: Tail different from above; head angle (laterally) more than 35°.....9

- 9a: Subdigital lamellae keeled.....10
- 9b: Subdigital lamellae smooth.....11

- 10a: All scales on body except chin shields, supralabials, and infralabials keeled; dorsal tubercles in each caudal segment not in contact, subcaudal scales not denticulate*Carinatogecko* Golubev and Szczerbak, 1981
- 10b: All body scales are not keeled; dorsal tubercles in each segment are in contact; subcaudal scales denticulate.....*Bunopus* Blanford, 1874

- 11a: Digit shape sub-angular, spine-shaped caudal tubercles do not contact each other in the whorls of a segment.....*Mediodactylus* Szczerbak and Golubev, 1977
- 11b: Digit shape strongly angular, low, or moderately high tail tubercles broadly contact each other across the dorsum of tail whorls and are surrounded by one to two smaller scales.....12

- 12a: Femoral pores present.....*Tenuidactylus* Szczerbak and Golubev, 1984
- 12b: Femoral pores absent.....*Cyrtopodion* Fitzinger, 1843

- 13a: Subcaudals arranged in single large, broad row, two strongly keeled tubercles on either side of each annulus.....*Parsigecko* Safaei-Mahroo, Ghaffari, and Anderson, 2016
- 13b: Tail scales uniform, homogenous, not keeled.....14

- 14a: Subdigital lamellae keeled.....*Tropicolotes* Peters, 1880
- 14b: Subdigital lamellae smooth.....*Microgecko* Nikolsky, 1907

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Farhang Torki earned his B.Sc. degree in Animal Biology from Lorestan University, Iran, and his M.Sc. degree in Animal Biosystematics from Razi University in Iran. During his B.Sc. studies, Farhang worked on histological and embryological methods, particularly as applied to the spermatogenesis and oogenesis of reptiles, and the herpetofauna of Lorestan Province. During his M.Sc. studies, he worked on the systematics of amphibians and reptiles of the southern and western Iranian Plateau and continued his developmental biology work in herpetology. Following his graduate work, Farhang established (2006) the Farhang Torki Ecology and Herpetology Center for Research (FTEHCR), the Farhang Torki Herpetology Museum (FTHM), and recently Biomatical Center for Researches (BMCR) in 2018. Currently, Farhang is studying the evolution and developmental biology, based on mathematical methods. Iran University of Medical Sciences supported his research during 2018.



Chiropterotriton chico García-Castillo, Rovito, Wake, and Parra-Olea 2017. El Chico Salamander is a state endemic species known only from Parque Nacional El Chico, at elevations from 2,400 to 3,050 m in pine-oak forest (Frost 2019). In the original description the authors noted that “it is unlikely to occur more widely, because surrounding areas have been extensively surveyed” (García-Castillo et al. 2017: 502). Also noted in the original description is that “this species was previously considered as conspecific with *C. multidentatus* and occurs in sympatry with *C. dimidiatus* and *Aquiloeurycea cephalica*. Likewise, *Isthmura bellii* has been collected very near sites where *C. chico* was once common..., but it is unknown if the two species occur in syntopy” (García-Castillo et al. 2017: 502). García-Castillo et al. (2017: 502–503) noted that this salamander was “incredibly abundant” when one of the authors of this paper (David B. Wake) visited the park in August of 1971, but that by the mid-1970s it had become “uncommon and then rare.” These authors also indicated (p. 503) that the decline of *C. chico* apparently is not related to habitat loss or disturbance. They concluded that this salamander should be judged to be Critically Endangered, based on the IUCN criteria B1 ab(v). Finally, García-Castillo et al. (2017: 503) noted that “Hidalgo includes an unusual region where two of the main mountain complexes of Mexico meet: the Trans-Mexican Volcanic Belt (TMVB) and Sierra Madre Oriental (SMO). These ranges are known to have a high degree of topographic, geologic and climatic variability [that] has promoted a high biodiversity... This is especially true for the herpetofauna from Hidalgo... which is the state with the highest number of species of *Chiropterotriton* (6), representing 37.5% of the described species of this genus.” Photo by Sean Rovito, courtesy of Mirna G. García-Castillo.



The herpetofauna of Hidalgo, Mexico: composition, distribution, and conservation status

¹Aurelio Ramírez-Bautista, ²Uriel Hernández-Salinas, ¹Raciel Cruz-Elizalde,
³Christian Berriozabal-Islas, ¹Israel Moreno-Lara, ⁴Dominic L. DeSantis, ⁵Jerry D. Johnson,
⁶Elí García-Padilla, ⁵Vicente Mata-Silva, and ^{7,8}Larry David Wilson

¹Laboratorio de Ecología de Poblaciones, Centro de Investigaciones Biológicas, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Km 4.5 Carretera Pachuca-Tulancingo, 42184 Mineral de La Reforma, Hidalgo, MEXICO ²Instituto Politécnico Nacional, CIIDIR Unidad Durango, Sigma 119, Fraccionamiento 20 de Noviembre II, Durango 34220, MEXICO ³Programa Educativo de Ingeniería en Biotecnología, Universidad Politécnica de Quintana Roo, Av. Arco Bicentenario, M 11, Lote 1119-33, Sm 255, 77500 Cancún, Quintana Roo, MEXICO ⁴Department of Biological and Environmental Sciences, Georgia College and State University, Milledgeville, Georgia 31061, USA ⁵Department of Biological Sciences, The University of Texas at El Paso, El Paso, Texas 79968-0500, USA ⁶Oaxaca de Juárez, Oaxaca 68023, MEXICO ⁷Centro Zamorano de Biodiversidad, Escuela Agrícola Panamericana Zamorano, Departamento de Francisco Morazán, HONDURAS ⁸1350 Pelican Court, Homestead, Florida 33035-1031, USA

Abstract.—The herpetofauna of Hidalgo, Mexico, is comprised of 203 species, including 42 anurans, 17 caudates, one crocodylian, 137 squamates, and six turtles. Here, the distribution of the herpetofaunal species are catalogued among the four recognized physiographic regions. The total number of species varies from 77 in the Mexican Plateau to 166 in the Sierra Madre Oriental. The individual species occupy from one to four regions (mean = 2.1). About 69% of the Hidalgo herpetofauna is found in only one or two of the four regions, which is of considerable conservation significance. The greatest number of single-region species occupies the Sierra Madre Oriental (25), followed by the Gulf Coastal Lowlands (15), the Trans-Mexican Volcanic Belt (6), and the Mexican Plateau (2). The Coefficient of Biogeographic Resemblance (CBR) indicates that the Sierra Madre Oriental and the Gulf Coastal lowlands share the most species (72), because of their adjacent geographic position and they contain a significant number of generalist species that occur in the Gulf lowlands of Mexico, southern USA, Central America, and/or South America. The two largest geographic regions in Hidalgo by area, Sierra Madre Oriental and Mexican Plateau, reflect opposite patterns in species richness (166 and 77 species, respectively) due to overall differences in the ecological characteristics between them. A similarity dendrogram based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) depicts two distinct clusters, one between the Sierra Madre Oriental and Gulf Coastal Lowlands and the other between the Mexican Plateau and Trans-Mexican Volcanic Belt. The former cluster reflects the two regions sharing a substantial number of herpetofaunal species that occur on the Gulf lowlands of North America and Central America, as well as a few that enter South America. The second cluster is due to the two montane regions being adjacent to one another and their ecological similarities. With respect to the distributional categories, the largest number of species is that of the country endemics (104 of 203), followed by non-endemics (92), state endemics (four), and non-natives (three). The principal environmental threats to the Hidalgo herpetofauna are deforestation, livestock, roads, pollution of water sources, cultural factors, and diseases. The conservation status of each native species was assessed by means of the SEMARNAT (NOM-059), IUCN, and EVS systems, of which the EVS system was the most useful. The Relative Herpetofaunal Priority (RHP) method was also used to designate the rank order significance of the physiographic regions and the highest values were found for the Sierra Madre Oriental. Most of the five protected areas in Hidalgo are located in the Trans-Mexican Volcanic Belt, which is only the second most important region from a conservation perspective. In addition, only 78 of the 200 native species found in Hidalgo are recorded in total from the five protected areas. Finally, a set of conclusions and recommendations are offered for the future protection of the Hidalgo herpetofauna.

Keywords. Anurans, caudates, crocodylians, physiographic regions, protected areas, protection recommendations, squamates, turtles

Resumen.—La herpetofauna de Hidalgo, México, consiste de 203 especies, incluyendo 42 anuros, 17 caudados, un cocodrilo, 137 escamosos, y seis tortugas. Catalogamos la distribución de las especies entre cuatro regiones fisiográficas aquí reconocidas. El número total de especies varía de 77 en la Altiplanicie Mexicana, a 166 en la Sierra Madre Oriental. Las especies individualmente ocupan de una a cuatro regiones ($x = 2.1$). Aproximadamente 69% de la herpetofauna de Hidalgo se encuentra en una o dos de las cuatro regiones, lo

Correspondence. ramibautistaa@gmail.com (ARB), uherndez3@gmail.com (UHS), cruzelizalde@gmail.com (RCE), christianberriozabal@gmail.com (CBI), izraa.miara150911@gmail.com (IML), dominic.desantis@gcsu.edu (DLD), jjohnson@utep.edu (JDJ), eligarcia_18@hotmail.com (EGP), vmata@utep.edu (VMS), bufodoc@aol.com (LDW)

cual es de importancia considerable para su conservación. El mayor número de especies que ocupan una sola región, se encuentra en la Sierra Madre Oriental (25), seguida por las Tierras Costeras del Golfo (15), la Faja Volcánica Transmexicana (6), y la Altiplanicie Mexicana (2). Una matriz de coeficiente de similitud biogeográfica (CSB), indica que la Sierra Madre Oriental y las Tierras Costeras del Golfo comparten la mayoría de las especies (72) debido a su proximidad geográfica y al número significativo de especies generalistas presentes en las Tierras Costeras del Golfo de México, sur de Estados Unidos, Centroamérica, y/o Suramérica. Con respecto a la superficie, las dos regiones más grandes de Hidalgo, la Sierra Madre Oriental y la Faja Volcánica Transmexicana reflejan relaciones opuestas sobre la riqueza específica (166 vs 77 especies, respectivamente) debido a las características ecológicas entre estas. Un dendrograma de similitud basado en el Método por Agrupamiento de Pares no Ponderado con Media Aritmética (MAPMA) revela dos agrupamientos; uno entre la Sierra Madre Oriental y las Tierras Costeras del Golfo, y el otro entre las dos regiones que comparten la Faja Volcánica Transmexicana. El primer grupo se debe a que las dos regiones comparten un número significativo de especies que ocurren en las tierras costeras del golfo de Norteamérica y Centroamérica, así como algunas especies que llegan a Sudamérica. El segundo grupo se debe al contacto de estas dos regiones y sus similitudes ecológicas. Con respecto a las categorías de distribución, el mayor número de especies está representado por las especies endémicas a México (104 de 203), seguido por las especies no endémicas (92), endémicas para el estado (cuatro), y las no nativas (tres). Las principales amenazas ambientales para la herpetofauna de Hidalgo son la deforestación, ganadería, carreteras, contaminación de fuentes de agua, factores culturales, y enfermedades. Calculamos el estatus de conservación de las especies nativas por medio de los sistemas de SEMARNAT (NOM-059), IUCN, y el EVS, de los cuales el EVS resultó ser el más útil. También utilizamos el método de Prioridad Herpetofaunística Relativa (PHR) para designar el rango de orden de importancia de las regiones fisiográficas y determinamos que los valores más altos pertenecen a la Sierra Madre Oriental. Examinamos las características de las cinco áreas protegidas en Hidalgo y determinamos que la mayoría de estas se encuentran en la Faja Volcánica Transmexicana, que es la segunda región más importante desde la perspectiva de conservación. También determinamos que solamente 78 de las 200 especies nativas registradas en Hidalgo, se encuentran en estas cinco áreas protegidas. Finalmente, establecemos un conjunto de conclusiones y recomendaciones para la futura protección de la herpetofauna de Hidalgo.

Palabras Claves. Anuros, caudados, estatus de conservación, cocodrilidos, regiones fisiográficas, áreas protegidas, recomendaciones de protección, escamosos, tortugas

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“One reason we’ve failed to recognize the damage we’re doing we’ve assumed it’s fine to use our atmosphere as an open sewer.”

—Al Gore (2017)

Introduction

Hidalgo is an eastern Mexican state located at the confluence of four major physiographic regions in the country, i.e., the Gulf Coastal Plain, occupying a small eastern portion of the state; the Sierra Madre Oriental, extending across the majority of the eastern sector of the state; the Trans-Mexican Volcanic Belt, traversing the southern portion of the state; and the Mexican Plateau, occupying the central and northwestern regions of the state (Fig. 1). Hidalgo is bounded to the north by San Luis Potosí and Querétaro, to the east by Veracruz and Puebla, to the south by Tlaxcala, and to the west by México.

With a surface area of 20,813 km², Hidalgo is the 26th largest state in Mexico among the 31 that are recognized (<http://cuentame.inegi.org.mx/monografias/informacion/hgo/>).

The 2015 population figure was 2,858,359, ranking the state as the 17th most populous (<http://cuentame.inegi.org.mx/monografias/informacion/hgo/>). The population density of Hidalgo is 140 people/km², ranking 8th in the country (<http://cuentame.inegi.org.mx/monografias/informacion/hgo/>).

The highest elevation in the state of 3,380 m is that of Cerro la Peñuela, located in the southeastern-most corner of Hidalgo in the municipality of Almoloya (<http://cuentame.inegi.org.mx/monografias/informacion/hgo/>). Even though much of the state of Hidalgo is mountainous, the heights of these mountains are much less imposing than those of the neighboring state of Puebla (see Woolrich-Piña et al. 2017). The highest mountain in Puebla, Pico de Orizaba, is 5,747 m in elevation, which is 1.7 times the height of Cerro la Peñuela. Two of the other highest mountains in Mexico also are partially located in Puebla (Woolrich-Piña et al. 2017). Thus, Hidalgo would be expected to have a smaller total herpetofauna than Puebla and also support fewer endemic species, both at the national and state levels. An examination of these hypotheses is undertaken in this paper.

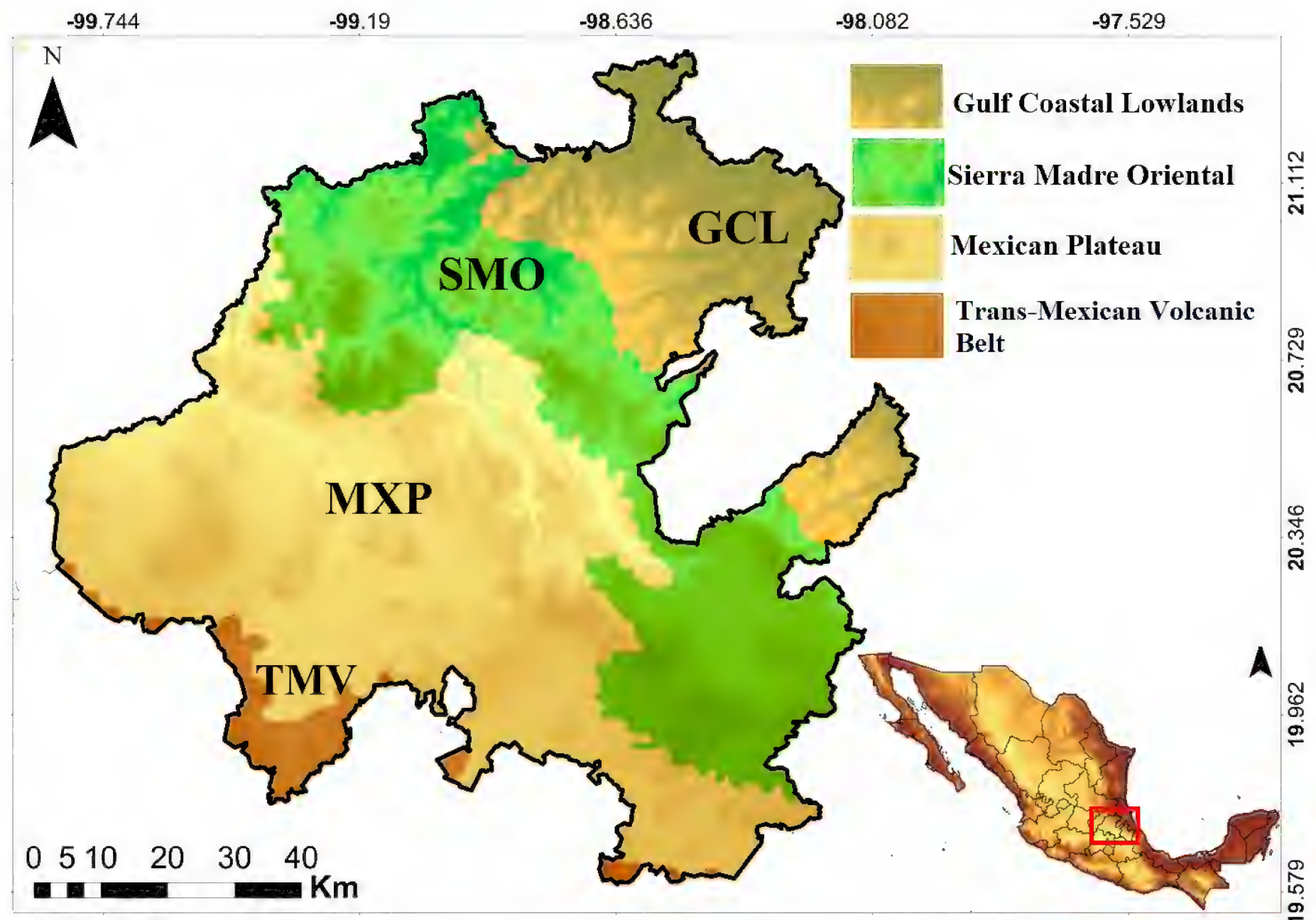


Fig. 1. Physiographic regions of Hidalgo, Mexico. Abbreviations: GCL = Gulf Coastal Lowlands; SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt.

Materials and Methods

Our Taxonomic Position

We follow the same taxonomic position in this paper as explained in previous works on other portions of Mesoamerica (Johnson et al. 2015a,b; Mata-Silva et al. 2015; Terán-Juárez et al. 2016; Woolrich-Piña et al. 2016, 2017; Nevárez-de los Reyes et al. 2016; Cruz-Sáenz et al. 2016; Gonzalez-Sánchez et al. 2017; Lazcano et al. 2019). Johnson et al. (2015a) can be consulted for a detailed statement of this position, with special reference to the subspecies concept.

System for Determining Distributional Status

The same system developed by Alvarado-Díaz et al. (2013) for the herpetofauna of Michoacán was employed here to ascertain the distributional status of members of the herpetofauna of Hidalgo. Subsequently, Mata-Silva et al. (2015), Johnson et al. (2015a), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016, 2017), Nevárez-de los Reyes et al. (2016), Cruz-Sánchez et al. (2017), González-Sánchez et al. (2017), and Lazcano et al. (2019) also used this system, which consists of the following four categories: SE = endemic to Hidalgo; CE = endemic to Mexico; NE = not endemic to Mexico; NN = non-native in Mexico.

Systems for Determining Conservation Status

To assess the conservation status of the herpetofauna of Hidalgo, this survey employed the same systems (i.e., SEMARNAT, IUCN, and EVS) as used by Alvarado-Díaz et al. (2013), Mata-Silva et al. (2015), Johnson et al. (2015a), Terán-Juárez et al. (2016), Woolrich-Piña et al. (2016, 2017), Nevárez-de los Reyes et al. (2016), Cruz-Sánchez et al. (2017), González-Sánchez et al. (2017), and Lazcano et al. (2019). Detailed descriptions of these three systems appear in the earlier papers of this series (e.g., Alvarado-Díaz et al. 2013; Johnson et al. 2015a; Mata-Silva et al. 2015) and do not need to be repeated here.

The Mexican Conservation Series

The Mexican Conservation Series (MCS) was initiated in 2013, with a study of the herpetofauna of Michoacán (Alvarado-Díaz et al. 2013), as a part of a set of five papers designated as the Special Mexico Issue published in *Amphibian & Reptile Conservation*. The basic format of the entries in the MCS was established in that paper, i.e., providing an examination of the composition, physiographic distribution, and conservation status of the herpetofauna of a given Mexican state or group of states. Two years later, the MCS continued with papers

on the herpetofauna of Oaxaca (Mata-Silva et al. 2015) and Chiapas (Johnson et al. 2015a). In the ensuing year, three entries in the MCS appeared, those on Tamaulipas (Terán-Juárez et al. 2016), Nayarit (Woolrich-Piña et al. 2016), and Nuevo León (Nevárez-de los Reyes et al. 2016). Finally, three entries on Jalisco (Cruz-Sáenz et al. 2017), the Mexican Yucatan Peninsula (González-Sánchez et al. 2017), and Puebla (Woolrich-Piña et al. 2017) appeared in 2017, and one on Coahuila was published recently (Lazcano et al. 2019). Thus, this paper on the herpetofauna of Hidalgo is the eleventh entry in this series.

Physiography and Climate

Physiographic Regions

The distribution of the herpetofauna of Hidalgo is analyzed using the classification system of physiographic regions (= physiographic provinces) of INEGI (2000) and CONABIO (2008). According to these studies, these consist of four regions, which are briefly described below.

Gulf Coastal Lowlands (GCL). This province belongs to the Neotropical Region (Morrone 2001) and extends from the San Fernando River in the state of Tamaulipas to the Candelaria River located in the Yucatan Peninsula. This region covers the northern portion of the states of Quintana Roo, Campeche, Tabasco, and Veracruz, and small portions of Tamaulipas, eastern San Luis Potosí, northeastern Hidalgo, northern Puebla, northeastern Oaxaca, and Campeche. Specifically, in the state of Hidalgo, this physiographic region is located in the municipalities of San Felipe Orizatlán and Huejutla (Sánchez-Rojas and Bravo-Cadena 2017). This region is located between 25°52'17.02"N, -94°04'11.48"W, and 20°55'36.56"N, -90°18'06.7"W, at elevations spanning 18–1,200 m (Espinosa et al. 2008). Mean annual precipitation varies between 1,000 and 2,000 mm, while the average annual temperature for this physiographic province is 21.2 °C. Dominant vegetation types include tropical evergreen forest, scrub, subdeciduous forest, and tropical dry forest (CONABIO 2008).

Sierra Madre Oriental (SMO). This region is located parallel to the Gulf coastal region of Mexico, which is connected to the Central Plateau and the Trans-Mexican Volcanic Belt. The SMO belongs to the Neotropical Realm and embraces 2.84% of the country (Morrone 2001; CONABIO 2008). This province is composed mostly of sedimentary and metamorphic rocks from the Cretaceous and Jurassic, which makes this province a complex area from a geological perspective (CONABIO 2008). The SMO encompasses part of southern Zacatecas, central and eastern Jalisco, southern Michoacán, Querétaro, and northeastern Hidalgo (CONABIO 2008). In the state of Hidalgo, this province extends into the municipalities

of Huehuetla and Tenango de Doria, Calnali, Molango, Tlanchinol, Lolotla, Chapulhuacán, Pisaflores, Tepehuacán, Xochicoatlán, and Eloxochitlán (CONABIO 2008; Ramírez-Bautista et al. 2014; Sánchez-Rojas and Bravo-Cadena 2017). The northern extent of the SMO lies at 25°36'23.13"N, -100°17'38.99"W, and the southern limit lies at 17°28'45.86"N, -96°04'34.85"W. Elevation within the SMO in Hidalgo ranges from 100–3,300 m (CONABIO 2008; Sánchez-Rojas and Bravo-Cadena 2017). Mean annual precipitation varies considerably, ranging from 400–800 mm in the montane cloud forests of the northern region of the municipality of Tlanchinol (Ramírez-Bautista et al. 2014). The temperature in the montane environments ranges from 4–28 °C, and from 10–40 °C in the temperate valleys during winter and summer, respectively. The average annual temperature is 17.4 °C. On the wet slopes, the dominant vegetation communities are coniferous forest (28%), oak forest (26%), and cloud forest (8%); and the vegetation is represented by xerophilous scrub (16%) in the dry region (CONABIO 2008).

Trans-Mexican Volcanic Belt (TMV). The TMV belongs to the Neotropical Region (Morrone 2001; CONABIO 2008), and is a volcanic arc located in the central part of Mexico. The TMV has an east-west orientation, extending from the state of Veracruz (Gulf of Mexico) to the state of Nayarit (Pacific Ocean; Ferrusquía-Villafranca 2007; CONABIO 2008). This belt is formed by a set of volcanoes of different ages, from Miocene to Plio-Pleistocene, aligned within 19°31'54.81"N, -98°37'42.45"W and 21°53'40.02"N, -105°36'09.80"W. The region occupies 8% of Mexico's surface area, ranging in elevation from 1,000–5,700 m. In Hidalgo the TMV reaches a high point of 2,004 m. Within the TMV lies the Sierra de Pachuca, which includes the municipalities of Mineral del Monte, Mineral El Chico, Huasca de Ocampo, Atotonilco El Grande, and a portion of Tulancingo (Ramírez-Bautista et al. 2014). The mean annual precipitation varies from 581–2,236 mm, and the mean annual temperature is 15.3 °C (Suárez-Mota et al. 2014). Natural vegetation communities are represented primarily by coniferous forest (31%) and oak forest (28%), with the remainder composed of pastures, subalpine scrub, cloud forest, and farmland. Arid portions of the region are dominated by xerophilous scrub, while sub-humid areas contain tropical dry forest.

Mexican Plateau (MXP). This region is within the more inclusive Nearctic Region (Morrone 2001; CONABIO 2008). This plateau extends through the central zone of Mexico between 1,700–4,000 m, and it is located between the Sierra Madre Occidental and Sierra Madre Oriental. Portions of the MXP fall within the boundaries of Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Puebla, San Luis Potosí, Tlaxcala, and Zacatecas. This region is confined within



No. 1. *Craugastor decoratus* (Taylor 1942). The Adorned Robber Frog is distributed from southern Tamaulipas, eastern San Luis Potosí, adjacent northern Querétaro, northern Hidalgo, and adjacent central Veracruz and northern Puebla, Mexico (Frost 2019). This individual was found at La Gargantilla, in the municipality of Pisaflores. Wilson et al. (2013b) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has been considered as Vulnerable by the IUCN, and as Special Protection (Pr) by SEMARNAT. *Photo by Daniel Lara-Tufiño.*



No. 2. *Charadrahyla taeniopus* (Günther 1901). The Porthole Treefrog occurs “on the Atlantic slopes of the Sierra Madre Oriental from north-eastern Hidalgo southward through northern Puebla to central Veracruz, Mexico” (Frost 2019). This individual was located at San Mateo, in the municipality of Acaxochitlán. Wilson et al. (2013b) assessed its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been judged as Vulnerable by the IUCN, and it is placed in the Threatened (A) category by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 3. *Dryophytes euphorbiaceus* (Günther 1858). The Southern Highland Treefrog ranges from the “highlands of southern Mexico (central Veracruz, eastern Hidalgo, Tlaxcala, and southeastern Puebla to mountains of Oaxaca, including those south of Oaxaca City)” (Frost 2019). This individual was encountered at San Mateo, in the municipality of Acaxochitlán. Wilson et al. (2013b) calculated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has been considered as Near Threatened by the IUCN, but this species is not listed by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 4. *Dryophytes plicatus* (Brocchi 1877). The Ridged Treefrog is distributed in the Sierra Madre Oriental and the Cordillera Volcánica along the southern edge of the Mexican Plateau (Michoacán, Morelos, México, D.F., Tlaxcala, Puebla, Veracruz, and Hidalgo) [Frost 2019]. This individual was found at Agua Zarca, in the municipality of Tenango de Doria. Wilson et al. (2013b) calculated its EVS as 11, placing it in the middle portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and SEMARNAT lists this treefrog as Threatened (A). *Photo by Uriel Hernández-Salinas.*

Table 1. Monthly minimum, mean (in parentheses), maximum, and annual temperature data (in °C) for the physiographic regions of Hidalgo, Mexico. Localities for each of the regions and their elevations are as follows: Gulf Coastal Plain—Huehuetla (420 m); Sierra Madre Oriental—Tlanchinol (1,700 m); Trans-Mexican Volcanic Belt—Tepeapulco (2,530 m); Mexican Plateau—Zimapan (1,763 m). Data taken from <http://www.worldclim.org/bioclim> (accessed 19 March 2018).

Physiographic Region	January	February	March	April	May	June	July	August	September	October	November	December	Annual
Gulf Coastal Lowlands	11.5 (17.2) 22.9	12.7 (18.5) 24.2	15.6 (21.3) 27.0	18.2 (23.9) 29.6	20.0 (25.7) 31.4	19.8 (25.5) 31.3	19.2 (24.5) 29.8	19.4 (24.7) 30.0	19.7 (24.5) 29.3	17.0 (22.3) 27.6	14.8 (20.1) 25.4	12.7 (18.0) 23.3	16.7 (21.2) 27.7
Sierra Madre Oriental	7.0 (13.8) 20.5	8.2 (15.0) 21.8	10.9 (17.6) 24.4	12.7 (19.4) 26.2	13.8 (20.5) 27.3	13.1 (19.8) 26.6	11.7 (18.8) 26.0	11.7 (18.8) 26.0	11.3 (18.4) 25.6	9.8 (16.9) 24.2	8.4 (15.5) 22.8	7.1 (14.3) 21.5	10.5 (17.4) 24.4
Trans Mexican Volcanic Belt	4.9 (12.2) 19.5	5.9 (13.2) 20.5	8.2 (15.5) 22.8	9.8 (17.1) 24.4	10.6 (17.9) 25.2	10.1 (17.4) 24.7	7.8 (16.5) 25.6	7.8 (16.6) 25.3	7.4 (16.1) 24.9	6.2 (14.9) 23.6	4.9 (13.7) 22.4	3.9 (12.6) 21.3	7.3 (15.3) 23.4
Mexican Plateau	6.0 (13.1) 20.2	7.1 (14.3) 21.4	9.6 (16.8) 23.9	11.3 (18.5) 25.6	12.3 (19.5) 26.6	11.6 (18.8) 25.9	9.5 (17.8) 26.1	9.5 (17.8) 26.1	9.1 (17.4) 25.7	7.8 (16.1) 24.3	6.4 (14.7) 23.0	5.4 (13.7) 21.9	8.8 (18.5) 24.2

24°39'53.31"N, -101°54'04.92"W and 19°31'54.81"N, -98°37'42.45"W, encompassing a total surface area of 601,882 m². The climate is dry, arid, and relatively cold; the mean annual temperature is 18.5 °C, with a nocturnal mean of -5 °C and a diurnal mean of 25 °C. Dominant vegetation communities include thorn scrub and xerophilous scrub. Mean annual precipitation is < 200 mm across all vegetation communities. The MXP is situated in the central and western portions of Hidalgo, in the municipalities of Mineral de la Reforma, Actopan, Ixmiquilpan, Zimapan, and Huichapan (Ramírez-Bautista et al. 2014).

Climature

Temperature. The monthly minimum, mean, and maximum temperatures for a single locality for each of the four recognized physiographic regions in Hidalgo are shown in Table 1. The elevations for these localities vary from 420 m at Huehuetla in the GCL to 2,530 m at Tepeapulco in the TMX.

The mean annual temperature is highest at Huehuetla (elevation 420 m) in the GCL at 21.2 °C, followed by Zimapan (elevation 1,763 m) in the MXP at 18.5 °C, and Tlanchinol (elevation 1,700 m) in the SMO at 17.4 °C, with the lowest mean temperature of 15.3 °C at Tepeapulco (elevation 2,530 m) in the TMV.

In the four physiographic regions in Hidalgo, the minimum annual temperatures range from 11.0–16.1 °C lower than the maximum annual temperatures (Table 1). The mean minimum monthly temperatures peak during May and reach their lowest levels in December or January. The mean maximum monthly temperatures are highest in May or July and are lowest in January (Table 1). The mean monthly temperatures are highest in May and lowest in January (Table 1).

Precipitation. As expected, monthly precipitation is lowest during the dry season in either December or February, and highest during the rainy season in either July or September (Table 2). The data in Table 2 indicate that 79.2–94.3% of the annual precipitation occurs during the rainy season from May to October. Annual rainfall varies from 537.5 mm on the Mexican Plateau to 1,790.7 mm in the Gulf Coastal Lowlands (Table 2).

Recent Literature on the Hidalgo Herpetofauna

Historically, most information on the herpetofauna of the state was derived from regional studies or those from specific localities (see Ramírez-Bautista et al. [2014] and Lemos-Espinal and Dixon [2016] for a detailed list of previous studies in the state). In 2010, a checklist of the state’s herpetofauna (Ramírez-Bautista et al. 2010) included a total of 173 species (54 amphibians and 119 reptiles), and subsequently Ramírez-Bautista et al. (2014) published an updated checklist with 183

Table 2. Monthly and annual precipitation data (in mm) for the physiographic regions of Hidalgo, Mexico. Localities for each of the regions and their elevations are as follows: Gulf Coastal Plain—Huehuetla (420 m); Sierra Madre Oriental—Tlanchinol (1,700 m); Trans Mexican Volcanic Belt—Tepeapulco (2,530 m); Mexican Plateau—Zimapan (1,763 m). Data taken from <http://www.worldclim.org/bioclim> (accessed 19 March 2018). The shaded area indicates the months of the rainy season.

Physiographic Region	Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Gulf Coastal Lowlands	51.9	51.8	52.0	73.4	126.6	268.9	261.2	239.8	334.8	186.3	89.8	54.2	1,790.7
Sierra Madre Oriental	21.0	19.6	20.6	36.8	61.0	140.3	135.4	130.7	179.2	92.7	36.2	20.6	894.1
Trans Mexican Volcanic Belt	10.3	8.7	11.6	25.7	58.8	111.5	131.7	110.5	100.3	49.0	14.1	7.8	1,379.3
Mexican Plateau	11.6	9.5	12.2	26.7	51.9	90.3	91.9	80.2	93.6	47.3	14.5	7.8	537.5

species (53 amphibians and 130 reptiles), along with the first comprehensive analysis of the state's species richness, diversity, and distribution, and an evaluation of the conservation status and potential threats to the persistence of Hidalgo's herpetofauna. Lemos-Espinal and Smith (2015) provided an additional checklist of the herpetofauna of Hidalgo with a total of 175 species, eight fewer than the number reported by Ramírez-Bautista et al. (2014). Lemos-Espinal and Dixon (2016) reported the same figure (175 species). Finally, in 2017 two chapters on the herpetofauna of the state were published in a book entitled *Biodiversidad del Estado de Hidalgo*. The chapter on amphibians by Goyenechea Mayer-Goyenechea et al. (2017) listed 53 species, and the chapter on reptiles by Manríquez-Morán et al. (2017) listed 130 species; both were the same numbers given by Ramírez-Bautista et al. (2014). The assessment here provides the most current species composition for the state following the latest taxonomic changes.

Composition of the Herpetofauna

Families

The herpetofauna of Hidalgo is represented by 37 families, including nine anuran, three salamander, one crocodylian, 22 squamate, and two turtle families (Table 3). This total figure is 62.7% of the 59 herpetofaunal families recorded from Mexico (Wilson et al. 2013a,b). No caecilians are registered in the state. Of the amphibian families, 86.4% of the species are placed in the families Bufonidae, Eleutherodactylidae, Hylidae, Ranidae, and Plethodontidae (Tables 4–5). Among the reptile families, 75.7% of the species are allocated to the families

Anguidae, Dactyloidae, Phrynosomatidae, Colubridae, Dipsadidae, Natricidae, and Viperidae (Tables 4–5).

Genera

Ninety-six herpetofaunal genera are represented in Hidalgo, including 20 anuran, seven salamander, one crocodylian, 65 squamate, and three turtle genera (Table 3). These 96 taxa comprise 45.7% of the 210 genera recorded from Mexico (Wilson et al. 2013a,b). Among the amphibians, the most species-rich genera are *Craugastor* (five species), *Eleutherodactylus* (five), *Lithobates* (five), and *Chiropterotriton* (nine). Among the reptile genera, the most speciose are *Sceloporus* (13), *Thamnophis* (nine), *Crotalus* (nine), and *Norops* (five).

Species

The herpetofauna of Hidalgo is composed of 203 species, including 42 anurans, 17 salamanders, one crocodylian, 137 squamates, and six turtles (Table 3). The current numbers of native species in these five groups in Mexico are, respectively, 248, 151, three, 865, and 51 (J. Johnson, unpub.). The 203 species in Hidalgo comprise 15.4% of the 1,318 species in the entire Mexican herpetofauna (J. Johnson, unpub., 26 June 2019).

The one state sharing a common border with Hidalgo examined thus far in the Mexican Conservation Series is Puebla (Woolrich-Piña et al. 2017), the herpetofauna of which consists of 267 species or 1.3 times the richness of Hidalgo (203). This comparative figure somewhat resembles the relative areas of the two states. The surface area of Puebla is 34,306 km² (Woolrich-Piña et al. 2017) and that of Hidalgo, as noted above, is 20,813 km²; thus, Puebla is 1.6

Table 3. Composition of the native and non-native herpetofauna of Hidalgo, Mexico.

Order	Families	Genera	Species
Anura	9	20	42
Caudata	3	7	17
Subtotal	12	27	59
Crocodylia	1	1	1
Squamata	22	65	137
Testudines	2	3	6
Subtotal	25	69	144
Total	37	96	203



No. 5. *Rheohyla miotympanum* (Cope, 1863). The Small-eared Treefrog occupies the “highlands of Nuevo León and Coahuila (Sierra Madre Oriental) to Guanajuato (Sierra Santa Rosa), Hidalgo, and Oaxaca, adjacent Veracruz, and central Chiapas in eastern and central Mexico” (Frost 2019). This individual was located at San Mateo, in the municipality of Acaxochitlán. Wilson et al. (2013b) determined its EVS as 9, placing it at the upper limit of the low vulnerability category. Its conservation status has been considered as Near Threatened by the IUCN, but this species is not listed by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 6. *Sarcohyla robertsoni* (Taylor 1940). Roberts’ Treefrog occupies “the Sierra Madre Oriental in eastern Mexico (Puebla and Hidalgo)” (Frost 2019). This individual was encountered in Zoquizoquiapan, in the municipality of Metztlán. Wilson et al. (2013b) assessed its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been considered as Endangered by the IUCN, and it is placed in the Threatened (A) category by SEMARNAT. *Photo by Raquel Hernández-Austria.*



No. 7. *Lithobates johnei* (Blair, 1965). John’s Frog is distributed from “southeastern San Luis Potosí, eastern Hidalgo, and northern Puebla, Mexico” (Frost 2019). This individual was found at Río Blanco, in the municipality of Huehuetla. Wilson et al. (2013b) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Endangered by the IUCN, and it is placed in the Endangered (P) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 8. *Lithobates montezumae* (Baird 1854). The Montezuma Leopard Frog ranges from San Luis Potosí, Querétaro, Jalisco, and eastern Durango south to the southeastern edge of the Mexican Plateau in Tlaxcala, Puebla, Hidalgo, Ciudad de México, and Veracruz, Mexico (Frost 2019). This individual was discovered at El Huemac, in the municipality of Tezontepec de Aldama. Wilson et al. (2013b) estimated its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and it is allocated to the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

Table 4. Distribution of the amphibians, crocodylians, squamates, and turtles of Hidalgo, Mexico, by physiographic region. Abbreviations: SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt; and GCL = Gulf Coastal Lowlands. See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Physiographic regions of Hidalgo				Number of regions occupied
	SMO	MXP	TMV	GCL	
Anura (42 species)					
Bufonidae (6 species)					
<i>Anaxyrus punctatus</i>	+	+	+		3
<i>Incilius marmoreus</i> *			+	+	2
<i>Incilius nebulifer</i>	+		+		2
<i>Incilius occidentalis</i> *	+	+	+		3
<i>Incilius valliceps</i>	+	+		+	3
<i>Rhinella horribilis</i>	+	+		+	3
Craugastoridae (5 species)					
<i>Craugastor augusti</i>	+		+		2
<i>Craugastor berkenbuschii</i> *				+	1
<i>Craugastor decoratus</i> *	+			+	2
<i>Craugastor mexicanus</i> *	+			+	2
<i>Craugastor rhodopis</i> *	+			+	2
Eleutherodactylidae (5 species)					
<i>Eleutherodactylus cystignathoides</i>	+			+	2
<i>Eleutherodactylus guttillatus</i>	+	+	+		3
<i>Eleutherodactylus longipes</i> *	+		+		2
<i>Eleutherodactylus nitidus</i> *	+		+		2
<i>Eleutherodactylus verrucipes</i> *	+		+		2
Hylidae (15 species)					
<i>Bromeliahyla dendroscarta</i> *	+			+	2
<i>Charadrahyla taeniopus</i> *	+				1
<i>Dryophytes arenicolor</i>	+	+			2
<i>Dryophytes euphorbiaceus</i> *	+		+	+	3
<i>Dryophytes eximius</i> *	+	+	+		3
<i>Dryophytes plicatus</i> *	+	+	+		3
<i>Rheohyla miotympanum</i> *	+	+		+	3
<i>Sarcohyla arborescandens</i> *	+	+	+		3
<i>Sarcohyla bistincta</i> *	+	+	+		3
<i>Sarcohyla charadricola</i> *	+	+			2
<i>Sarcohyla robertsororum</i> *	+	+			2
<i>Scinax staufferi</i>				+	1
<i>Smilisca baudinii</i>	+			+	2
<i>Tlalocohyla picta</i>	+			+	2
<i>Trachycephalus vermiculatus</i>	+			+	2
Leptodactylidae (2 species)					
<i>Leptodactylus fragilis</i>	+			+	2
<i>Leptodactylus melanonotus</i>	+	+		+	3
Microhylidae (1 species)					
<i>Hypopachus variolosus</i>			+	+	2
Ranidae (5 species)					
<i>Lithobates berlandieri</i>	+	+	+	+	4
<i>Lithobates catesbeianus</i> ***			+	+	2
<i>Lithobates johnei</i> *	+			+	2
<i>Lithobates montezumae</i> *	+	+	+		3
<i>Lithobates spectabilis</i> *	+	+	+		3
Rhinophrynidae (1 species)					
<i>Rhinophrynus dorsalis</i>	+			+	2
Scaphiopodidae (2 species)					
<i>Scaphiopus couchii</i>	+				1
<i>Spea multiplicata</i>		+	+		2
Caudata (17 species)					

Table 4 (continued). Distribution of the amphibians, crocodylians, squamates, and turtles of Hidalgo, Mexico, by physiographic region. Abbreviations: SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt; and GCL = Gulf Coastal Lowlands. See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Physiographic regions of Hidalgo				Number of regions occupied
	SMO	MXP	TMV	GCL	
Ambystomatidae (1 species)					
<i>Ambystoma velasci</i> *	+	+	+		3
Plethodontidae (15 species)					
<i>Aquiloemycea cephalica</i> *	+		+		2
<i>Bolitoglossa platydactyla</i> *				+	1
<i>Chiropterotriton arboreus</i> *	+		+		2
<i>Chiropterotriton chico</i> **			+		1
<i>Chiropterotriton chiropterus</i> *		+	+		2
<i>Chiropterotriton chondrostega</i> *	+		+		2
<i>Chiropterotriton dimidiatus</i> **	+	+	+		3
<i>Chiropterotriton magnipes</i> *			+		1
<i>Chiropterotriton mosaueri</i> **	+	+	+		3
<i>Chiropterotriton multidentatus</i> *	+	+	+		3
<i>Chiropterotriton terrestris</i> **	+		+		2
<i>Isthmura bellii</i> *	+	+	+		3
<i>Isthmura gigantea</i> *	+				1
<i>Pseudoeurycea altamontana</i> *			+		1
<i>Pseudoeurycea leprosa</i> *	+	+	+		3
Salamandridae (1 species)					
<i>Notophthalmus meridionalis</i>	+			+	2
Crocodylia (1 species)					
Crocodylidae (1 species)					
<i>Crocodylus moreletii</i>				+	1
Squamata (136 species)					
Anguidae (5 species)					
<i>Abronia taeniata</i> *	+	+	+		3
<i>Barisia imbricata</i> *	+	+	+		3
<i>Gerrhonotus infernalis</i>	+	+			2
<i>Gerrhonotus liocephalus</i>	+		+		2
<i>Gerrhonotus ophiurus</i> *	+			+	2
Corytophanidae (3 species)					
<i>Basiliscus vittatus</i>				+	1
<i>Corytophanes hernandezii</i>	+			+	2
<i>Laemactus serratus</i>	+			+	2
Dactyloidae (5 species)					
<i>Norops laeviventris</i>	+				1
<i>Norops lemurinus</i>	+			+	2
<i>Norops naufragus</i> *	+			+	2
<i>Norops petersii</i>	+			+	2
<i>Norops sericeus</i>	+			+	2
Dibamidae (1 species)					
<i>Anelytropsis papillosus</i> *	+			+	2
Eublepharidae (1 species)					
<i>Coleonyx elegans</i>	+				1
Gekkonidae (1 species)					
<i>Hemidactylus frenatus</i> ***				+	1
Iguanidae (1 species)					
<i>Ctenosaura acanthura</i>				+	1
Phrynosomatidae (14 species)					
<i>Phrynosoma orbiculare</i> *	+	+	+		3
<i>Sceloporus aeneus</i> *	+	+	+		3
<i>Sceloporus bicanthalis</i> *			+		1
<i>Sceloporus cyanogenys</i>	+				1
<i>Sceloporus grammicus</i>	+	+	+		3

Table 4 (continued). Distribution of the amphibians, crocodylians, squamates, and turtles of Hidalgo, Mexico, by physiographic region. Abbreviations: SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt; and GCL = Gulf Coastal Lowlands. See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Physiographic regions of Hidalgo				Number of regions occupied
	SMO	MXP	TMV	GCL	
<i>Sceloporus megalepidurus</i> *	+	+	+		3
<i>Sceloporus minor</i> *	+	+	+		3
<i>Sceloporus mucronatus</i> *		+	+		2
<i>Sceloporus parvus</i> *	+	+			2
<i>Sceloporus scalaris</i> *	+	+	+		3
<i>Sceloporus serrifer</i>	+			+	2
<i>Sceloporus spinosus</i> *	+	+	+		3
<i>Sceloporus torquatus</i> *	+	+	+		3
<i>Sceloporus variabilis</i>	+	+		+	3
Scincidae (2 species)					
<i>Plestiodon lynxe</i> *	+	+	+		3
<i>Plestiodon tetragrammus</i>	+				1
Sphenomorphidae (2 species)					
<i>Scincella gemmingeri</i> *	+	+		+	3
<i>Scincella silvicola</i> *	+		+	+	3
Teiidae (2 species)					
<i>Aspidoscelis gularis</i>	+			+	2
<i>Holcosus amphigrammus</i> *	+			+	2
Xantusiidae (4 species)					
<i>Lepidophyma flavimaculatum</i>	+			+	2
<i>Lepidophyma gaigeae</i> *	+		+		2
<i>Lepidophyma occulor</i> *	+			+	2
<i>Lepidophyma sylvaticum</i> *	+			+	2
Xenosauridae (3 species)					
<i>Xenosaurus mendozai</i> *	+				1
<i>Xenosaurus newmanorum</i> *	+				1
<i>Xenosaurus tzacualtipantecus</i> *	+				1
Boidae (1 species)					
<i>Boa imperator</i>	+			+	2
Colubridae (32 species)					
<i>Coluber constrictor</i>				+	1
<i>Conopsis biserialis</i> *	+		+		2
<i>Conopsis lineata</i> *	+	+	+		3
<i>Conopsis nasus</i> *	+	+	+		3
<i>Drymarchon melanurus</i>	+	+		+	3
<i>Drymobius chloroticus</i>	+			+	2
<i>Drymobius margaritiferus</i>	+			+	2
<i>Ficimia hardyi</i> *	+	+			2
<i>Ficimia olivacea</i> *			+	+	2
<i>Ficimia streckeri</i>	+			+	2
<i>Lampropeltis annulata</i>		+			1
<i>Lampropeltis mexicana</i> *	+	+			2
<i>Lampropeltis polyzona</i> *	+			+	2
<i>Lampropeltis ruthveni</i> *		+	+		2
<i>Leptophis diplotropis</i> *				+	1
<i>Leptophis mexicanus</i>	+			+	2
<i>Masticophis flagellum</i>	+				1
<i>Masticophis mentovarius</i>		+	+		2
<i>Masticophis schotti</i>		+	+	+	3
<i>Mastigodryas melanolomus</i>	+			+	2
<i>Oxybelis aeneus</i>	+			+	2
<i>Pantherophis emoryi</i>	+	+		+	3
<i>Pituophis catenifer</i>	+				1
<i>Pituophis deppei</i> *	+	+	+		3
<i>Pseudelaphe flavirufa</i>		+		+	2

Table 4 (continued). Distribution of the amphibians, crocodylians, squamates, and turtles of Hidalgo, Mexico, by physiographic region. Abbreviations: SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt; and GCL = Gulf Coastal Lowlands. See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Physiographic regions of Hidalgo				Number of regions occupied
	SMO	MXP	TMV	GCL	
<i>Salvadora bairdi</i> *	+	+			2
<i>Salvadora grahamiae</i>	+		+	+	3
<i>Senticolis triaspis</i>	+		+	+	3
<i>Spilotes pullatus</i>	+			+	2
<i>Tantilla bocourti</i> *	+	+	+	+	4
<i>Tantilla rubra</i>	+				1
<i>Trimorphodon tau</i> *	+		+		2
Dipsadidae (27 species)					
<i>Adelphicos quadrivirgatum</i>	+			+	2
<i>Amastridium sapperi</i>	+			+	2
<i>Chersodromus rubriventris</i> *	+			+	2
<i>Coniophanes fissidens</i>	+			+	2
<i>Coniophanes imperialis</i>				+	1
<i>Coniophanes piceivittis</i>				+	1
<i>Diadophis punctatus</i>	+	+	+		3
<i>Geophis latifrontalis</i> *	+				1
<i>Geophis lorancai</i>	+				1
<i>Geophis mutitorques</i> *	+	+	+		3
<i>Geophis semidoliatus</i> *	+	+	+		3
<i>Geophis turbidus</i> *	+				1
<i>Hypsiglena jani</i>	+	+			2
<i>Hypsiglena tanzeri</i> *	+				1
<i>Imantodes cenchoa</i>	+			+	2
<i>Imantodes gemmistratus</i>	+			+	2
<i>Leptodeira maculata</i> *	+			+	2
<i>Leptodeira septentrionalis</i>	+			+	2
<i>Ninia diademata</i>	+			+	2
<i>Pliocercus elapoides</i>	+			+	2
<i>Rhadinaea decorata</i>	+				1
<i>Rhadinaea gaigeae</i> *			+		1
<i>Rhadinaea hesperia</i> *			+		1
<i>Rhadinaea marcellae</i> *	+				1
<i>Rhadinaea quinquelineata</i> *	+	+	+	+	4
<i>Sibon nebulatus</i>	+			+	2
<i>Tropidodipsas sartorii</i>	+	+		+	3
Elapidae (2 species)					
<i>Micrurus diastema</i>	+				1
<i>Micrurus tener</i>	+	+		+	3
Leptotyphlopidae (3 species)					
<i>Epictia wynni</i> *				+	1
<i>Rena dulcis</i>		+			1
<i>Rena myopica</i> *	+			+	2
Natricidae (13 species)					
<i>Nerodia rhombifer</i>	+			+	2
<i>Storeria dekayi</i>	+				1
<i>Storeria hidalgoensis</i> *	+				1
<i>Storeria storerioides</i> *		+	+		2
<i>Thamnophis cyrtopsis</i>	+	+	+		3
<i>Thamnophis eques</i>	+	+	+		3
<i>Thamnophis marcianus</i>	+		+	+	3
<i>Thamnophis melanogaster</i> *	+	+			2
<i>Thamnophis proximus</i>	+			+	2
<i>Thamnophis pulchrilatus</i> *			+	+	2
<i>Thamnophis scalaris</i> *	+		+		2

Table 4 (continued). Distribution of the amphibians, crocodylians, squamates, and turtles of Hidalgo, Mexico, by physiographic region. Abbreviations: SMO = Sierra Madre Oriental; MXP = Mexican Plateau; TMV = Trans-Mexican Volcanic Belt; and GCL = Gulf Coastal Lowlands. See text for descriptions of these regions. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Physiographic regions of Hidalgo				Number of regions occupied
	SMO	MXP	TMV	GCL	
<i>Thamnophis scaliger</i> *		+	+		2
<i>Thamnophis sumichrasti</i> *	+	+			2
Sibynophiidae (1 species)					
<i>Scaphiodontophis annulatus</i>				+	1
Typhlopidae (1 species)					
<i>Indotyphlops braminus</i> ***	+	+	+		3
Viperidae (13 species)					
<i>Agkistrodon taylori</i> *	+			+	2
<i>Bothrops asper</i>	+			+	2
<i>Crotalus aquilus</i> *	+	+	+		3
<i>Crotalus atrox</i>	+	+		+	3
<i>Crotalus intermedius</i> *	+		+		2
<i>Crotalus molossus</i>	+	+	+		3
<i>Crotalus polystictus</i> *	+	+	+		3
<i>Crotalus ravus</i> *	+	+	+		3
<i>Crotalus scutulatus</i>	+	+	+		3
<i>Crotalus totonacus</i> *	+			+	2
<i>Crotalus triseriatus</i> *	+	+	+		3
<i>Metlapilcoatlus nummifer</i> *	+				1
<i>Ophryacus smaragdinus</i> *	+				1
Testudines (6 species)					
Emydidae (2 species)					
<i>Terrapene mexicana</i> *	+				1
<i>Trachemys venusta</i>				+	1
Kinosternidae (4 species)					
<i>Kinosternon herrerae</i> *	+			+	2
<i>Kinosternon hirtipes</i>	+		+		2
<i>Kinosternon integrum</i> *	+	+	+		3
<i>Kinosternon scorpioides</i>				+	1

times the size of Hidalgo. Therefore, the state area/species richness ratio for Hidalgo is 102.5 compared to 128.5 for Puebla.

Patterns of Physiographic Distribution

Here, four physiographic regions in Hidalgo are recognized (Fig. 1), and the occurrence of the members of the herpetofauna among these four regions are documented in Table 4 and summarized in Table 5.

The total numbers of species in each of these four regions vary from a low of 77 in the Mexican Plateau (MXP) to a high of 166 in the Sierra Madre Oriental (SMO). The intermediate figures are 85 for the Trans-Mexican Volcanic Belt and 95 for the Gulf Coastal Lowlands. Interestingly, the number of species recorded from the Sierra Madre Oriental is about 1.7 to 2.2 times those in the three other regions in the state. The herpetofauna of the SMO comprises 81.8% of that of the entire state (203 species).

As expected, the largest proportions of the species by broader herpetofaunal groups are found in the SMO (Table 5), including 36 of 42 anurans (85.7%), 12 of 17

salamanders (70.6%), 114 of 137 squamates (83.2%), and four of six turtles (66.7%).

As noted above, the numbers of species in the other three regions are approximately half of that found in the SMO (Table 5). Of these three regions, the largest number of species (95) is found in the Gulf Coastal Lowlands, including 22 of 42 anurans (52.4%), two of 17 salamanders (11.8%), one of one crocodylian (100%), 67 of 137 squamates (48.9%), and three of six turtles (50.0%). The next largest number of species in these three regions (85) is found in the Trans-Mexican Volcanic Belt, including 20 of 42 anurans (47.6%), 14 of 17 salamanders (82.4%), 49 of 137 squamates (35.8%), and two of six turtles (33.3%). Finally, the smallest number of species (77) is registered on the Mexican Plateau, including 18 of 42 anurans (42.9%), seven of 17 salamanders (41.2%), 51 of 137 squamates (37.2%), and one of six turtles (16.7%).

The members of the Hidalgo herpetofauna inhabit from one to four of the four physiographic regions, as follows: one (48; 23.6%); two (93; 46.0%); three (59; 29.2%); and four (three; 1.5%). The average regional



No. 9. *Ambystoma velasci* (Dugès 1888). The Plateau Tiger Salamander ranges from northwestern Chihuahua south along the eastern slope of the Sierra Madre Occidental and southern Nuevo León to Hidalgo in the Sierra Madre Oriental, west to Zacatecas, and south into the Transverse Volcanic range of central Mexico (Frost 2019). This individual was located in Parque Nacional El Chico, in the municipality of Mineral del Chico. Wilson et al. (2013b) determined its EVS as 10, placing it at the lower limit of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and it has been placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 10. *Aquiloeurycea cephalica* (Cope 1865). The Chunky False Brook Salamander is distributed in “the Transverse Volcanic Range in Ciudad de México and states of Veracruz, Hidalgo, México, Puebla, and Morelos” (Frost 2019). This individual was found in the municipality of Tlanchinol. Wilson et al. (2013b) assessed its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Near Threatened by the IUCN, and as occupying the Threatened (A) category by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 11. *Bolitoglossa platydactyla* (Gray 1831). The Broad-footed Salamander occurs from “southern Tamaulipas and eastern San Luis Potosí south through Hidalgo to southern Veracruz, Puebla, Oaxaca, and extreme northeastern Chiapas, Mexico” (Frost 2019). This individual was found at Cececamel in the municipality of San Felipe Orizatlán. Wilson et al. (2013b) ascertained its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status had been judged as Near Threatened by the IUCN, and SEMARNAT has placed it in the Special Protection (Pr) category. *Photo by Cristian Raúl Olvera-Olvera.*



No. 12. *Isthmura belli* (Gray 1850). Bell's Salamander is found in “southern Tamaulipas, Tlaxcala, Hidalgo and the Sierra Madre del Sur of Guerrero, Mexico, and west and north to southern Nayarit and southern Zacatecas” (Frost 2019). This individual was encountered at Las Coas, in the municipality of Tlahuiltepa. Wilson et al. (2013b) calculated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has been considered as Vulnerable by the IUCN, and as Threatened (A) by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

Table 5. Summary of distribution occurrence of herpetofaunal families in Hidalgo, Mexico, by physiographic province. See Table 4 for explanation of abbreviations.

Family	Number of species	Distributional occurrence			
		SMO	MXP	TMV	GCL
Bufonidae	6	5	4	4	3
Craugastoridae	5	4	—	1	4
Eleutherodactylidae	5	5	1	4	1
Hylidae	15	14	8	5	7
Leptodactylidae	2	2	1	—	2
Microhylidae	1	—	—	1	1
Ranidae	5	4	3	4	3
Rhinophrynidae	1	1	—	—	1
Scaphiopodidae	2	1	1	1	—
Subtotal	42	36	18	20	22
Ambystomatidae	1	1	1	1	—
Plethodontidae	15	10	6	13	1
Salamandridae	1	1	—	—	1
Subtotal	17	12	7	14	2
Total	59	48	25	34	24
Crocodylidae	1	—	—	—	1
Subtotal	1	—	—	—	1
Anguidae	5	5	3	3	1
Corytophanidae	3	2	—	—	3
Dactyloidae	5	5	—	—	4
Dibamidae	1	1	—	—	1
Eublepharidae	1	1	—	—	—
Gekkonidae	1	—	—	—	1
Iguanidae	1	—	—	—	1
Phrynosomatidae	14	12	10	10	2
Scincidae	2	2	1	1	—
Sphenomorphidae	2	2	1	1	2
Teiidae	2	2	—	—	2
Xantusiidae	4	4	—	1	3
Xenosauridae	3	3	—	—	1
Subtotal	44	39	15	16	21
Boidae	1	1	—	—	1
Colubridae	32	24	14	12	18
Dipsadidae	27	23	6	6	15
Elapidae	2	2	1	—	1
Leptotyphlopidae	3	1	1	—	2
Natricidae	13	10	6	7	4
Sibynophiidae	1	—	—	—	1
Typhlopidae	1	1	1	1	—
Viperidae	13	13	7	7	4
Subtotal	93	75	36	33	46
Emydidae	2	1	—	—	1
Kinosternidae	4	3	1	2	2
Subtotal	6	4	1	2	3
Total	144	118	52	51	71
Sum Total	203	166	77	85	95

occupancy is 2.1, which means that, on average, each individual species occupies only about half of the physiographic regions found in the state.

A sizable proportion of the herpetofauna is distributed in one or two regions (141, or 69.5% of the total). This proportion is very close to that seen for Puebla (68.5%; Woolrich-Piña et al. 2017); in the case of Puebla, however, there are six regions instead of the four found in Hidalgo.

The number of species found in a single region range from three (in the MXP) to 25 (in the SMO). The 25 single-region species in the SMO are:

*Charadrahyla taeniopus**
Scaphiopus couchii
*Isthmura gigantea**
Norops laeviventris
Coleonyx elegans
Sceloporus cyanogenys
Plestiodon tetragrammus
*Xenosaurus mendozai**
*Xenosaurus newmanorum**
*Xenosaurus tzacualtipantecus**
Masticophis flagellum
Pituophis catenifer
Tantilla rubra

Table 6. Pair-wise comparison matrix of Coefficient of Biogeographic Resemblance (CBR) data of herpetofaunal relationships for the four physiographic regions in Hidalgo, Mexico. Underlined values = number of species in each region; upper triangular matrix values = species in common between two regions; and lower triangular matrix values = CBR values. The formula for this algorithm is $CBR = 2C/N_1 + N_2$ (Duellman 1990), where C is the number of species in common to both regions, N_1 is the number of species in the first region, and N_2 is the number of species in the second region. See Fig. 6 for the UPGMA dendrogram produced from the CBR data.

	Sierra Madre Oriental	Mexican Plateau	Trans-Mexican Volcanic Belt	Gulf Coastal Lowlands
Sierra Madre Oriental	<u>166</u>	67	66	72
Mexican Plateau	0.55	<u>77</u>	53	16
Trans-Mexican Volcanic Belt	0.53	0.65	<u>85</u>	13
Gulf Coastal Lowlands	0.55	0.19	0.14	<u>95</u>

*Geophis latifrontalis**
*Geophis lorancai**
*Geophis turbidus**
*Hypsiglena tanzeri**
Rhadinaea decorata
*Rhadinaea marcellae**
Micrurus diastema
Storeria dekayi
*Storeria hidalgoensis**
*Metlapilcoatlus nummifer**
*Ophryacus smaragdinus**
*Terrapene mexicana**

Fourteen of the 25 SMO single-region species (56.0%) are Mexican endemics (indicated by asterisks); the remainder are non-endemic species.

The 15 single-region species in the GCL are:

*Craugastor berkenbuschii**
Scinax staufferi
*Bolitoglossa platydactyla**
Crocodylus moreletii
Basiliscus vittatus
*Hemidactylus frenatus****
Ctenosaura acanthura
Coluber constrictor
*Leptophis diplotropis**
Coniophanes imperialis
Coniophanes piceivittis
*Epictia wynni**
Scaphiodontophis annulatus
Trachemys venusta
Kinosternon scorpioides

Four of the 15 GCL single-region species are Mexican endemics (single asterisk), one is a non-native (triple asterisk), and the remainder are non-endemic species.

The six single-region species in the TMV are:

*Chiropterotriton chico***
*Chiropterotriton magnipes**
*Pseudoeurycea altamontana**
*Sceloporus bicanthalis**
*Rhadinaea gaigeae**
*Rhadinaea hesperia**

Five of the six TMV single-region species are Mexican endemics and one is a state endemic.

The two single-region species in the MXP are:

Lampropeltis annulata
Rena dulcis

Both of these species are non-endemics.

In summary, of the 48 single-region species in Hidalgo, 23 are Mexican endemics, one is a state endemic, 23 are non-endemics, and one is a non-native. Of the four physiographic regions, the SMO is of the greatest conservation importance given that it houses the greatest overall number of species (166), the greatest number of single-region species (25), and the largest number of country endemics (14).

A Coefficient of Biogeographic Resemblance (CBR) matrix was created for studying the herpetofaunal similarity relationships among the four physiographic regions in Hidalgo (Table 6) and those data were used to construct a UPGMA dendrogram. The SMO contains the greatest species richness (166 species) and the MXP the least (77 species). The mean species richness value for all four areas is 105.5. The number of shared species between each of the regional pairs ranges from a high of 72 between SMO and GCL to a low of 13 between TMV and GCL. The mean value of shared species among all four regions is 47.8. The lowest number of shared species between the TMV and the GCL (13 species) was expected because these two regions are situated on opposite ends of Hidalgo, are not connected geographically (being completely separated by the



No. 13. *Isthmura gigantea* (Taylor 1939). The Giant False Brook Salamander ranges “in the La Joya-Jalapa region of Veracruz and into northeastern Hidalgo, Mexico” (Frost 2019). This individual was encountered at Chilijapa, in the municipality of Tepehuacan de Guerrero. Wilson et al. (2013b) determined its EVS as 16, placing it in the middle portion of the high vulnerability category. Its conservation status has been considered as Critically Endangered by the IUCN, but this species is not listed by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 14. *Barisia imbricata* (Wiegmann, 1828). The Transvolcanic Alligator Lizard ranges in the Trans-Mexican Volcanic Belt and the Sierra Madre Oriental, in the states of México, Ciudad de México, Querétaro, Hidalgo, Jalisco, Puebla, Oaxaca, Michoacán, Morelos, and Tlaxcala. This individual was found at Puenteillas in the municipality of Singuilucan. Wilson et al. (2013b) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has been assessed as Least Concern by the IUCN, and it has been placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Cristian Raúl Olvera-Olvera.*



No. 15. *Gerrhonotus ophiurus* Cope 1867. This alligator lizard occurs in the Mexican states of Hidalgo, Veracruz, San Luis Potosí, Querétaro, Michoacán, and Puebla (Ramírez-Bautista et al. 2014). This individual was encountered at El Demañi, in the municipality of Tlahuiltepa. Wilson et al. (2013a) assessed its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, but this species is not listed by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 16. *Norops naufragus* (Campbell, Hillis, and Lamar 1989). The Hidalgo Anole is found only in the states of Hidalgo and Puebla in Mexico (Ramírez-Bautista et al. 2014). This individual was found at Cuatatlán, in the municipality of Tlanchinol. Wilson et al. (2013a) ascertained its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been considered as Vulnerable by the IUCN, and it is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

SMO and the MXP), and are environmentally different on an elevational scale. The GCL, with an elevational range from near sea level to 1,200 m, contains tropical evergreen forest and subhumid formations of scrublands to tropical dry forests. On the other hand, the TMV with a limited geographic area within Hidalgo contains humid, semihumid, and subhumid vegetation in montane environments at elevations from 1,000 m in large sloping river valleys to 3,400 m on volcanic peaks. The SMO and the GCL share the most species (72), which also was not unexpected because they are directly adjacent to each other in Hidalgo, and the tropical lowland environments of the GCL ascend into the mountainous habitats of the SMO. The pairwise comparisons of regions aligned in order from highest to lowest species richness (underlined values) and their corresponding numbers of shared species (in parentheses) are:

SMO 166: GCL (72), MXP (67), TMV (66)

GCL 95: SMO (72), MXP (16), TMV (13)

TMV 85: SMO (66), MXP (53), GLC (13)

MXP 77: SMO (67), TMV (53), GLC (16)

In general, the pattern indicates how species richness values within each of the four biogeographic regions of Hidalgo equate to numbers of shared species among the other three regions. There is a higher correlation of species richness values to number of shared species between regions that are in contact with each other, but also observed correlations between regions that share similar ecological parameters. Interestingly, the two regions that share the most species (72) are a highland region (SMO) and a lowland region (GCL), which is probably due to the GCL containing many generalist species that can endure both montane and non-montane environments in low to moderate elevations. The fact that the GCL shares few species with the MXP and the TMV gives credibility to the premise that regions separated by ecological barriers will share fewer species than they will with regions in direct contact.

The following data show ranges and mean numbers of shared species (bold in parentheses) for each of the four regions that are arranged according to increasing species richness (underlined values) in each region:

Sierra Madre Oriental – SMO (166): 66–72 (**68.3**)

Gulf Coastal Lowlands – GCL (95): 13–72 (**33.6**)

Trans-Mexican Volcanic Belt – TMV (85): 13–66 (**44.0**)

Mexican Plateau – MXP (77): 16–67 (**45.3**)

The mean numbers of shared species compared to the species richness values in all four regions indicate that higher species richness in pairwise comparisons does not translate into higher reciprocal numbers when all regional pairs are totaled. The most apparently extreme example of this is the comparison between the SMO

and the GCL—which are 1st and 2nd in species richness, but 1st and last (4th) in mean numbers of shared species, respectively. The SMO also has higher mean numbers of shared species with TMV (44.0) and MXP (45.3), but if GCL (2nd in species richness, last in mean number of shared species) is removed from the tabulation, the three montane regions have even higher mean numbers of shared species. Specifically, the average number of shared species between the SMO, the TMV, and the MXP combined is **62.0** (calculated from Table 6).

Regarding area, the two largest geographic regions, the SMO and the MXP, reflect opposite relationships in species richness (166 vs. 77 species, respectively). The SMO contains more tropical, subtropical humid, and semihumid vegetation formations compared to the mostly subhumid environments in the MXP, in addition to being in direct contact with the second most species-rich region, the GCL, which shares the highest number of species in Hidalgo with the SMO. The GCL, the second most speciose region and third smallest region in the state, contains 10 more species than does the TMV, the smallest area by far that also contains less humid and semi-humid environments than does GCL. Also note that Hidalgo is a relatively small state in area (5th smallest of the 31 in Mexico), which undoubtedly affects species richness. As an example, the adjacent state of Puebla, which is slightly larger and contains two more physiographic regions than does Hidalgo, contains 267 species of amphibians and reptiles (Woolrich-Piña et al. 2017).

Based on the data in Table 6, a UPGMA dendrogram (Fig. 6) was created to depict the herpetofaunal similarity resemblance patterns in a hierarchical fashion among the four physiographic regions of Hidalgo (see map, Fig. 1). The dendrogram is composed of two distinct clusters; one comprising two montane regions (MXP and TMV) at the 0.65 level and the other containing one montane region (SMO) and the lowland region (GCL) at the 0.55 level. The two clusters connect together at the 0.39 level. Regions within both clusters are adjacent to each other and depict patterns of ecological similarity; and in the case of the SMO and the GCL, they share generalist species that primarily occur on the Gulf-facing side that ascends from the lowlands (the GCL) into the higher elevations in the SMO. Fifty-three of the 203 herpetofaunal species (26.2%) presently known from Hidalgo are shared only between the SMO and the GCL (Table 4), and many of them are wide-ranging species along the Gulf versant of Mexico, some of which also enter the USA, and/or Central America and South America (Wilson and Johnson 2010). Those 53 species also represent 73.6% of the 72 species shared among the SMO, the GCL, and other regions in Hidalgo. We also predict that other species now restricted to either the SMO or especially the GCL eventually will be discovered in both regions. In our opinion, the shared generalist species within the SMO and the GCL are the exclusive reason why the SMO clusters with the GCL instead of with the two other montane regions (MXP and TMV).



No. 17. *Phrynosoma orbiculare* (Linnaeus 1758). The Mountain Horned Lizard is known from the states of Chihuahua, Aguascalientes, Hidalgo, Querétaro, San Luis Potosí, Michoacán, Ciudad de México, Estado de México, Jalisco, Morelos, Tlaxcala, and Guanajuato (Ramírez-Bautista et al. 2014). This individual was located in Parque Nacional El Chico, in the municipality of Mineral del Chico. Wilson et al. (2013a) determined its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and as Threatened (A) by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 18. *Sceloporus bicanthalis* Smith 1937. The Transvolcanic Bunchgrass Lizard is distributed in the states of Hidalgo, México, Oaxaca, Puebla, and Veracruz (Ramírez-Bautista et al. 2014). This individual was found in the municipality of Mineral El Chico. Wilson et al. (2013a) calculated its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, but this species is not listed by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 19. *Sceloporus minor* Cope 1885. The Minor Scaly Lizard ranges into the states of Nuevo León, Zacatecas, San Luis Potosí, Tamaulipas, Querétaro, and Guanajuato (Ramírez-Bautista et al. 2014). This individual was located at La Mesa, in the Municipality of Zacualtipán. Wilson et al. (2013a) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, but this species is not listed by SEMARNAT. *Photo by Aarón García-Rosales.*



No. 20. *Lepidophyma occulor* Smith 1942. The Jalpan Tropical Night Lizard has a restricted distribution in adjacent areas of Querétaro, San Luis Potosí, and Hidalgo (Ramírez-Bautista et al. 2014). This individual came from Puerto Oscuro, in the municipality of Pisaflores. Wilson et al. (2013a) determined its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and it is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Daniel Lara-Tuñiño.*

Distribution Status Categorizations

The assessment of the distribution status of the members of the Hidalgo herpetofauna here uses the system developed by Alvarado-Díaz et al. (2013) and employed in all the other entries in the Mexican Conservation Series (see above). The categories in the system are non-endemic, country endemic, state endemic, and non-native, and data are presented in Table 7 and summarized in Table 8.

The numbers of species in each of the four categories, in decreasing order of size, are: country endemics, 104 (51.2%); non-endemics, 92 (45.3%); state endemics, four (2.0%); and non-natives, three (1.5%). As with the states of Michoacán (Alvarado-Díaz et al. 2013), Nayarit (Woolrich-Piña et al. 2016), Jalisco (Cruz-Sáenz et al. 2017), and Puebla (Woolrich-Piña et al. 2017), the greatest number of herpetofaunal species in Hidalgo lies within the country endemic category. The largest number falls within the non-endemic category in the

Table 7. Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Anaxyrus punctatus</i>	NE	L (5)	LC	NS
<i>Incilius marmoreus</i> *	CE	M (11)	LC	NS
<i>Incilius nebulifer</i>	NE	L (6)	LC	NS
<i>Incilius occidentalis</i> *	CE	M (11)	LC	NS
<i>Incilius valliceps</i>	NE	L (6)	LC	NS
<i>Rhinella horribilis</i>	NE	L (3)	NE	NS
<i>Craugastor augusti</i>	NE	L (8)	LC	NS
<i>Craugastor berkenbuschii</i> *	CE	H (14)	NT	Pr
<i>Craugastor decoratus</i> *	CE	H (15)	VU	Pr
<i>Craugastor mexicanus</i> *	CE	M (10)	LC	NS
<i>Craugastor rhodopis</i> *	CE	H (14)	VU	NS
<i>Eleutherodactylus cystignathoides</i>	NE	M (12)	LC	NS
<i>Eleutherodactylus gutttilatus</i>	NE	M (11)	LC	NS
<i>Eleutherodactylus longipes</i> *	CE	H (15)	VU	NS
<i>Eleutherodactylus nitidus</i> *	CE	M (12)	LC	NS
<i>Eleutherodactylus verrucipes</i> *	CE	H (16)	VU	Pr
<i>Bromeliohyala dendroscarta</i> *	CE	H (17)	CR	Pr
<i>Charadrahyla taeniopus</i> *	CE	M (13)	VU	A
<i>Dryophytes arenicolor</i>	NE	L (7)	LC	NS
<i>Dryophytes euphorbiaceus</i> *	CE	M (13)	NT	NS
<i>Dryophytes eximius</i> *	CE	M (10)	LC	NS
<i>Dryophytes plicatus</i> *	CE	M (11)	LC	A
<i>Rheohyla miotympanum</i> *	CE	L (9)	NT	NS
<i>Sarcohyala arborescandens</i> *	CE	M (11)	EN	Pr
<i>Sarcohyala bistincta</i> *	CE	L (9)	LC	Pr
<i>Sarcohyala charadricola</i> *	CE	H (14)	EN	A
<i>Sarcohyala robertsorum</i> *	CE	M (13)	EN	A
<i>Scinax staufferi</i>	NE	L (4)	LC	NS
<i>Smilisca baudinii</i>	NE	L (3)	LC	NS
<i>Tlalocohyla picta</i>	NE	L (8)	LC	NS
<i>Trachycephalus vermiculatus</i>	NE	L (4)	LC	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Leptodactylus fragilis</i>	NE	L (5)	LC	NS
<i>Leptodactylus melanonotus</i>	NE	L (6)	LC	NS
<i>Hypopachus variolosus</i>	NE	L (4)	LC	NS
<i>Lithobates berlandieri</i>	NE	L (7)	LC	Pr
<i>Lithobates catesbeianus</i> ***	NN	—	—	—
<i>Lithobates johnei</i> *	CE	H (14)	EN	P
<i>Lithobates montezumae</i> *	CE	M (13)	LC	Pr
<i>Lithobates spectabilis</i> *	CE	M (12)	LC	NS
<i>Rhinophrynus dorsalis</i>	NE	L (8)	LC	Pr
<i>Scaphiopus couchii</i>	NE	L (3)	LC	NS
<i>Spea multiplicata</i>	NE	L (6)	LC	NS
<i>Ambystoma velasci</i> *	CE	M (10)	LC	Pr
<i>Aquiloerycea cephalica</i> *	CE	H (14)	NT	A
<i>Bolitoglossa platydactyla</i> *	CE	H (15)	NT	Pr
<i>Chiropterotriton arboreus</i> *	CE	H (18)	CR	Pr
<i>Chiropterotriton chico</i> **	SE	H (18)	NE	NS
<i>Chiropterotriton chiropterus</i> *	CE	H (16)	CR	Pr
<i>Chiropterotriton chondrostega</i> *	CE	H (17)	EN	Pr
<i>Chiropterotriton dimidiatus</i> **	SE	H (17)	EN	Pr
<i>Chiropterotriton magnipes</i> *	CE	H (16)	CR	Pr
<i>Chiropterotriton mosaueri</i> **	SE	H (18)	CR	Pr
<i>Chiropterotriton multidentatus</i> *	CE	H (15)	EN	Pr
<i>Chiropterotriton terrestris</i> **	SE	H (18)	CR	NS
<i>Isthmura bellii</i> *	CE	M (12)	VU	A
<i>Isthmura gigantea</i> *	CE	H (16)	CR	NS
<i>Pseudoeurycea altamontana</i> *	CE	H (17)	EN	Pr
<i>Pseudoeurycea leprosa</i> *	CE	H (16)	VU	A
<i>Notophthalmus meridionalis</i>	NE	M (12)	EN	P
<i>Crocodylus moreletii</i>	NE	M (13)	LC	Pr
<i>Abronia taeniata</i> *	CE	H (15)	VU	Pr
<i>Barisia imbricata</i> *	CE	H (14)	LC	Pr
<i>Gerrhonotus infernalis</i>	NE	M (13)	LC	NS
<i>Gerrhonotus liocephalus</i>	NE	L (6)	LC	Pr
<i>Gerrhonotus ophiurus</i> *	CE	M (12)	LC	NS
<i>Basiliscus vittatus</i>	NE	L (7)	LC	NS
<i>Corytophanes hernandezii</i>	NE	M (13)	LC	Pr
<i>Laemactus serratus</i>	NE	L (8)	LC	Pr
<i>Norops laevis</i>	NE	L (9)	NE	NS
<i>Norops lemurinus</i>	NE	L (8)	NE	NS
<i>Norops naufragus</i> *	CE	M (13)	VU	Pr

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Norops petersii</i>	NE	L (9)	NE	NS
<i>Norops sericeus</i>	NE	L (8)	NE	NS
<i>Anelytropsis papillosus</i> *	CE	M (10)	LC	A
<i>Coleonyx elegans</i>	NE	L (9)	LC	A
<i>Hemidactylus frenatus</i> ***	NN	—	—	—
<i>Ctenosaura acanthura</i>	NE	M (12)	NE	Pr
<i>Phrynosoma orbiculare</i> *	CE	M (12)	LC	A
<i>Sceloporus aeneus</i> *	CE	M (13)	LC	NS
<i>Sceloporus bicanthalis</i> *	CE	M (13)	LC	NS
<i>Sceloporus cyanogenys</i>	NE	M (13)	NE	NS
<i>Sceloporus grammicus</i>	NE	L (9)	LC	Pr
<i>Sceloporus megalepidurus</i> *	CE	H (14)	VU	Pr
<i>Sceloporus minor</i> *	CE	H (14)	LC	NS
<i>Sceloporus mucronatus</i> *	CE	M (13)	LC	NS
<i>Sceloporus parvus</i> *	CE	H (15)	LC	NS
<i>Sceloporus scalaris</i> *	CE	M (12)	LC	NS
<i>Sceloporus serrifer</i>	NE	L (6)	LC	NS
<i>Sceloporus spinosus</i> *	CE	M (12)	LC	NS
<i>Sceloporus torquatus</i> *	CE	M (11)	LC	NS
<i>Sceloporus variabilis</i>	NE	L (5)	LC	NS
<i>Plestiodon lynxe</i> *	CE	M (10)	LC	Pr
<i>Plestiodon tetragrammus</i>	NE	M (12)	LC	NS
<i>Scincella gemmingeri</i> *	CE	M (11)	LC	Pr
<i>Scincella silvicola</i> *	CE	M (12)	LC	A
<i>Aspidoscelis gularis</i>	NE	L (9)	LC	NS
<i>Holcosus amphigrammus</i> *	CE	M (11)	NE	NS
<i>Lepidophyma flavimaculatum</i>	NE	L (8)	LC	Pr
<i>Lepidophyma gaigeae</i> *	CE	M (13)	VU	Pr
<i>Lepidophyma occulor</i> *	CE	H (14)	LC	Pr
<i>Lepidophyma sylvaticum</i> *	CE	M (11)	LC	Pr
<i>Xenosaurus mendozai</i> *	CE	H (16)	NE	NS
<i>Xenosaurus newmanorum</i> *	CE	H (15)	EN	Pr
<i>Xenosaurus tzacualtipantecus</i> *	CE	H (16)	NE	NS
<i>Boa imperator</i>	NE	M (10)	NE	NS
<i>Coluber constrictor</i>	NE	M (10)	LC	A
<i>Conopsis biserialis</i> *	CE	M (13)	LC	A
<i>Conopsis lineata</i> *	CE	M (13)	LC	NS
<i>Conopsis nasus</i> *	CE	M (11)	LC	NS
<i>Drymarchon melanurus</i>	NE	L (6)	LC	NS
<i>Drymobius chloroticus</i>	NE	L (8)	LC	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Drymobius margaritiferus</i>	NE	L (6)	NE	NS
<i>Ficimia hardyi</i> *	CE	M (13)	EN	NS
<i>Ficimia olivacea</i> *	CE	L (9)	NE	NS
<i>Ficimia streckeri</i>	NE	M (12)	LC	NS
<i>Lampropeltis annulata</i>	NE	M (12)	NE	NS
<i>Lampropeltis mexicana</i> *	CE	H (15)	LC	A
<i>Lampropeltis polyzona</i> *	CE	M (11)	NE	NS
<i>Lampropeltis ruthveni</i> *	CE	H (16)	NT	A
<i>Leptophis diplotropis</i> *	CE	H (14)	LC	A
<i>Leptophis mexicanus</i>	NE	L (6)	LC	A
<i>Masticophis flagellum</i>	NE	L (8)	LC	A
<i>Masticophis mentovarius</i>	NE	L (6)	LC	A
<i>Masticophis schotti</i>	NE	M (13)	LC	NS
<i>Mastigodryas melanolomus</i>	NE	L (6)	LC	NS
<i>Oxybelis aeneus</i>	NE	L (5)	NE	NS
<i>Pantherophis emoryi</i>	NE	M (13)	LC	NS
<i>Pituophis catenifer</i>	NE	L (9)	LC	NS
<i>Pituophis deppei</i> *	CE	H (14)	LC	A
<i>Pseudelaphe flavirufa</i>	NE	M (10)	LC	NS
<i>Salvadora bairdi</i> *	CE	H (15)	LC	Pr
<i>Salvadora grahamiae</i>	NE	M (10)	LC	NS
<i>Senticolis triaspis</i>	NE	L (6)	LC	NS
<i>Spilotes pullatus</i>	NE	L (6)	NE	NS
<i>Tantilla bocourti</i> *	CE	L (9)	LC	NS
<i>Tantilla rubra</i>	NE	L (5)	LC	Pr
<i>Trimorphodon tau</i> *	CE	M (13)	LC	NS
<i>Adelphicos quadrivirgatum</i>	NE	M (10)	LC	Pr
<i>Amastridium sapperi</i>	NE	M (10)	LC	NS
<i>Chersodromus rubriventris</i> *	CE	H (14)	EN	Pr
<i>Coniophanes fissidens</i>	NE	L (7)	NE	NS
<i>Coniophanes imperialis</i>	NE	L (8)	LC	NS
<i>Coniophanes piceivittis</i>	NE	L (7)	LC	NS
<i>Diadophis punctatus</i>	NE	L (4)	LC	NS
<i>Geophis latifrontalis</i> *	CE	H (14)	DD	Pr
<i>Geophis lorancai</i>	CE	H (14)	NE	NS
<i>Geophis mutitorques</i> *	CE	M (13)	LC	Pr
<i>Geophis semidoliatus</i> *	CE	M (13)	LC	NS
<i>Geophis turbidus</i> *	CE	H (15)	NE	NS
<i>Hypsiglena jani</i>	NE	L (6)	NE	NS
<i>Hypsiglena tanzeri</i> *	CE	H (15)	DD	NS

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Imantodes cenchoa</i>	NE	L (6)	NE	Pr
<i>Imantodes gemmistratus</i>	NE	L (6)	NE	Pr
<i>Leptodeira maculata</i>	NE	L (7)	LC	Pr
<i>Leptodeira septentrionalis</i>	NE	L (8)	NE	NS
<i>Ninia diademata</i>	NE	L (9)	LC	NS
<i>Pliocercus elapoides</i>	NE	M (10)	LC	NS
<i>Rhadinaea decorata</i>	NE	L (9)	LC	NS
<i>Rhadinaea gaigeae</i> *	CE	M (12)	DD	NS
<i>Rhadinaea hesperia</i> *	CE	M (10)	LC	Pr
<i>Rhadinaea marcellae</i> *	CE	M (12)	EN	Pr
<i>Rhadinaea quinquelineata</i> *	CE	H (15)	DD	Pr
<i>Sibon nebulatus</i>	NE	L (5)	NE	NS
<i>Tropidodipsas sartorii</i>	NE	L (9)	LC	Pr
<i>Micrurus diastema</i>	NE	L (8)	LC	Pr
<i>Micrurus tener</i>	NE	M (11)	LC	NS
<i>Epictia wynni</i> *	CE	M (13)	NE	NS
<i>Rena dulcis</i>	NE	M (13)	LC	NS
<i>Rena myopica</i> *	CE	M (13)	LC	NS
<i>Nerodia rhombifer</i>	NE	M (10)	LC	NS
<i>Storeria dekayi</i>	NE	L (7)	LC	NS
<i>Storeria hidalgoensis</i> *	CE	M (13)	VU	NS
<i>Storeria storerioides</i> *	CE	M (11)	LC	NS
<i>Thamnophis cyrtopsis</i>	NE	L (7)	LC	A
<i>Thamnophis eques</i>	NE	L (8)	LC	A
<i>Thamnophis marcianus</i>	NE	M (10)	LC	A
<i>Thamnophis melanogaster</i> *	CE	H (15)	EN	A
<i>Thamnophis proximus</i>	NE	L (7)	LC	A
<i>Thamnophis pulchrilatus</i> *	CE	H (15)	LC	NS
<i>Thamnophis scalaris</i> *	CE	H (14)	LC	A
<i>Thamnophis scaliger</i> *	CE	H (15)	VU	A
<i>Thamnophis sumichrasti</i> *	CE	H (15)	LC	A
<i>Scaphiodontophis annulatus</i>	NE	M (11)	LC	NS
<i>Indotyphlops braminus</i> ***	NN	—	—	—
<i>Agkistrodon taylori</i> *	CE	H (17)	LC	A
<i>Bothrops asper</i>	NE	M (12)	NE	NS
<i>Crotalus aquilus</i> *	CE	H (16)	LC	Pr
<i>Crotalus atox</i>	NE	L (9)	LC	Pr
<i>Crotalus intermedius</i> *	CE	H (15)	LC	A
<i>Crotalus molossus</i>	NE	L (8)	LC	Pr
<i>Crotalus polystictus</i> *	CE	H (16)	LC	Pr

Table 7 (continued). Distributional and conservation status measures for members of the herpetofauna of Hidalgo, Mexico. Distributional Status: SE = endemic to Hidalgo; CE = endemic to country of Mexico; NE = not endemic to state or country; and NN = non-native. Environmental Vulnerability Score (taken from Wilson et al. 2013a,b): low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20). IUCN categorizations: CR = Critically Endangered; EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient; NE = Not Evaluated. SEMARNAT status: A = Threatened; P = Endangered; Pr = Special Protection; and NS = No Status. See text for explanations of the EVS, IUCN, and SEMARNAT rating systems.

Species	Distributional status	Environmental Vulnerability Category (Score)	IUCN categorization	SEMARNAT status
<i>Crotalus ravus</i> *	CE	H (14)	LC	A
<i>Crotalus scutulatus</i>	NE	M (11)	LC	Pr
<i>Crotalus totonacus</i> *	CE	H (17)	NE	NS
<i>Crotalus triseriatus</i> *	CE	H (16)	LC	NS
<i>Metlapilcoatlus nummifer</i> *	CE	M (13)	LC	A
<i>Ophryacus smaragdinus</i> *	CE	H (14)	NE	NS
<i>Terrapene mexicana</i> *	CE	H (19)	NE	NS
<i>Trachemys venusta</i>	NE	H (19)	VU	NS
<i>Kinosternon herrerae</i> *	CE	H (14)	NT	Pr
<i>Kinosternon hirtipes</i>	NE	M (10)	LC	Pr
<i>Kinosternon integrum</i> *	CE	M (11)	LC	Pr
<i>Kinosternon scorpioides</i>	NE	M (10)	NE	Pr

other states surveyed thus far: Oaxaca (Mata-Silva et al. 2015); Tamaulipas (Terán-Juárez et al. 2016); Nuevo León (Nevárez-de los Reyes et al. 2016); and Chiapas (Johnson et al. 2015a).

In the ten previous individual-state entries in the MCS, the numbers of state endemics varied considerably, from one in Nayarit and Nuevo León (Woolrich-Piña et al. 2016; Nevárez-de los Reyes et al. 2016) to a maximum of 93 in Oaxaca (Mata-Silva et al. 2015). The number of state endemics in Hidalgo is near the lower end of that range at four, and all of them are plethodontid salamanders in the genus *Chiropterotriton*: *C. chico*, *C. dimidiatus*, *C. mosaueri*, and *C. terrestris* (Table 7).

As noted in the introduction, we hypothesized that the number of endemic species should be greater for the state of Puebla than for Hidalgo. Woolrich-Piña et al. (2017) reported the number of country endemics for Puebla as 162 (60.7% of state total). As noted above, this figure for Hidalgo is 104, which is 51.2% of the state total. The number of state endemics, however, is the same for these two states, at four (Woolrich-Piña et al. 2017), which supports our hypothesis since Puebla has the greater total number of endemic species (166) than does Hidalgo (108).

Three non-native species occur in Hidalgo: *Lithobates catesbeianus*, *Hemidactylus frenatus*, and *Indotyphlops braminus*. Two of these three (*H. frenatus* and *I. braminus*) are the most widespread of the non-native species thus far recorded in the 11 entries in the Mexican Conservation Series (Woolrich-Piña et al. 2017), having been recorded, as of this paper, in 10 and 11 states, respectively.

Principal Environmental Threats

In this section we highlight the problems that we view as most significantly affecting the sustainability of Hidalgo's herpetofauna populations. The major threats include the increasing and unregulated clearing of forests for farming and livestock, construction of roads, the constant and increasing pollution of water bodies, emerging diseases, and strongly ingrained cultural factors (Ramírez-Bautista et al. 2014; Cruz-Elizalde et al. 2017).

Deforestation

The state of Hidalgo encompasses 903,502.5 ha used for livestock and agricultural activities (INEGI 2011). The area utilized for these activities, however, continues to increase, consequently eliminating ~1,200–5,102 ha of natural vegetation cover per year (SEMARNAT 2012). Hidalgo ranks from fifth to seventh among the 31 Mexican states in terms of deforestation rates (SEMARNAT 2012). Unfortunately, the loss of natural habitats affects both biological communities and human development in the region, since deforestation accelerates the loss of soils, increases water runoff, and accelerates the evaporation rates of water bodies that serve many local communities.

At the local level, increasing deforestation is driven primarily by agriculture. After farmers clear an area (often on pronounced slopes), they typically only use this land for one or two years. Once the terrain loses most of its top soil layers to erosion (Fig. 1), the site is abandoned in favor of clearing of a new area of native vegetation. For instance, in the SMO large trees are cut down and the lower vegetation is eliminated with



No. 21. *Lepidophyma sylvaticum* Taylor 1939. The Madrean Tropical Night Lizard is distributed in the Mexican states of Puebla, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas, and Veracruz (Ramírez-Bautista et al. 2014). This individual was located at La Cueva, in the municipality of Pisaflores. Wilson et al. (2013a) calculated its EVS as 11, placing it in the lower portion of the medium vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and it is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Daniel Lara-Tufiño.*



No. 22. *Xenosaurus mendozai* Nieto Montes de Oca, García Vázquez, Zúñiga-Vega, and Schmidt-Ballardo 2013. This individual was found at El Pinalito, in the municipality of Jacala de Ledezma. Wilson et al. (2013a) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has not been determined by the IUCN, and this species is not listed by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 23. *Xenosaurus newmanorum* Taylor 1949. Newman's Knob-scaled Lizard ranges from southeastern San Luis Potosí and extreme northern Hidalgo (Ramírez-Bautista et al. 2014). This individual was found at La Ameca, in the municipality of Pisaflores. Wilson et al. (2013a) assessed its EVS as 15, placing it in the lower portion of the high vulnerability category. Its conservation status has been considered as Endangered by the IUCN, and is allocated to the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 24. *Xenosaurus tzacualtipantecus* Woolrich-Piña and Smith 2012. The Zacualtipán Knob-scaled Lizard is limited to the Sierra Madre Oriental in the states of Hidalgo and Veracruz (Ramírez-Bautista et al. 2014). This individual came from La Mojonera, in the municipality of Zacualtipán. Wilson et al. (2013a) calculated its EVS as 17, placing it in the middle portion of the high vulnerability category. Its conservation status has been considered as Near Threatened by the IUCN, but this species is not listed by SEMARNAT. *Photo by Lia Victoria Berriozabal-Varela.*



Fig. 2. Gulf Coastal Lowlands. Riparian vegetation in the vicinity of Achiquihuitla in the municipality of Atlapexco. *Photo by Cristian Raúl Olvera-Olvera.*



Fig. 4. Mexican Plateau. Vegetation in the vicinity of Santa Monica in the municipality of Metztitlán. *Photo by Cristian Raúl Olvera-Olvera.*

controlled fires (Fig. 2). The removal of the arboreal layer affects a diversity of species that are dependent on specific microclimatic parameters. Some examples of herpetofaunal taxa involved are salamanders of the genera *Aquiloerycea*, *Chiropterotriton*, and *Isthmura*, anurans of the genera *Craugastor*, *Eleutherodactylus*, *Charadrahyla*, and *Plectrohyla*, lizards of the genera *Abronia*, *Norops*, *Corytophanes*, *Laemactus*, *Lepidophyma*, and *Xenosaurus*, and snakes of the genera *Boa*, *Spilotes*, *Metlapilcoatlus*, *Bothrops*, and *Ophryacus* (Cruz-Elizalde et al. 2017).

Livestock

Similar to agricultural deforestation, livestock ranching also involves vegetation removal for short-term exploitation. Livestock activities are associated with the destruction of thousands of ha of pristine forest. The soils in these pastures are prone to erosion and can only support one or two years of cattle grazing. Ranchers are then forced to look for new sites to clear at the expense of the natural ecosystems (Ramírez-Bautista et al. 2014). As a testimony to this crisis, in regions such as SMO and GCL, pasturelands have increased dramatically. Originally, these areas were covered with cloud forests and tropical forests (Fig. 3). The semiarid region in the state is not exempt from deforestation either, and goats are the main concern. Goat herders take their animals



Fig. 3. Sierra Madre Oriental. Panoramic view in the vicinity of Diego Mateo inside the Parque Nacional El Chico in the municipality of Mineral del Chico. *Photo by Cristian Raúl Olvera-Olvera.*

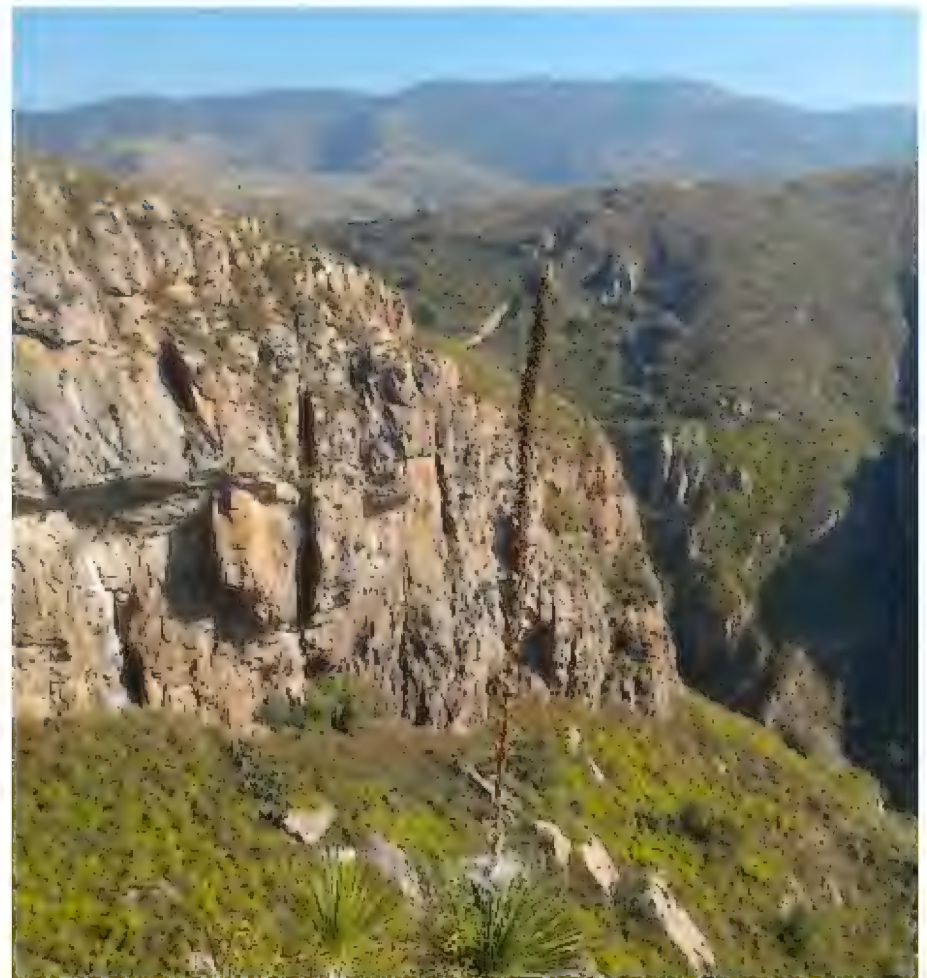


Fig. 5. Trans-Mexican Volcanic Belt. Sierra de Pachuca. Xerophilic scrub in Cerro de San Cristobal in the municipality of Pachuca. *Photo by Paola Lazcano-Juárez.*

to feed in areas covered with shrubs, destroying the slow-growth plants such as cacti and agaves, and in turn leading to erosion of the fragile soil (Ramírez-Bautista et al. 2014; Magno-Benítez et al. 2016).

Roads

Road infrastructure is important for the economic and social growth in the state. However, as is becoming more evident, this development brings adverse consequences to biodiversity (Puc Sánchez et al. 2013). Specifically, roads act as physical barriers for many amphibian and reptile species and reduce connectivity between populations. Vehicle-induced mortality or “roadkill” is one of the most visible effects of roads, as many herpetofauna cross busy roadways due to migration or

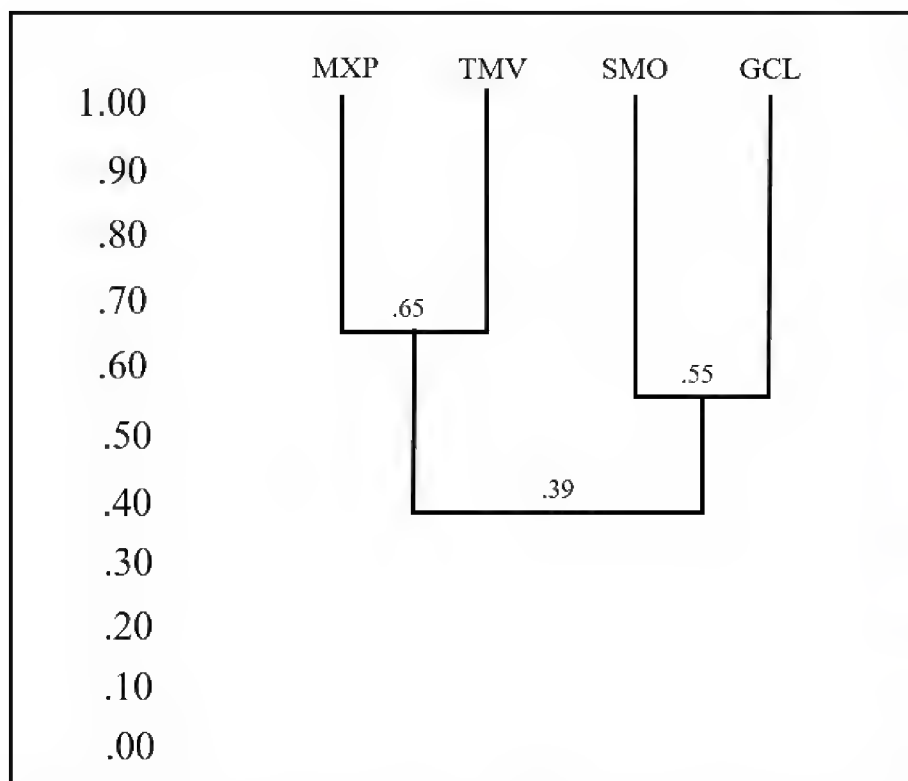


Fig. 6. A UPGMA generated dendrogram illustrating the similarity relationships of species richness among the herpetofauna in the four physiographic regions of Hidalgo (based on the data in Table 6). Similarity values were calculated values using Duellman's (1990) Coefficient of Biogeographic Resemblance (CBR).

dispersal, or use paved roads for basking (Figs. 4–5).

Pollution of water bodies

Continuous human population growth in Hidalgo (SEMARNAT 2012) and the lack of urban development plans have exacerbated the improper disposal of waste products and, consequently, have affected water sources such as rivers in the MXP, SMO, and GCL. Additionally, many fields used to produce vegetables in the western region of the state have been irrigated with sewage water, which, unfortunately, contaminates the soils and local water sources. The sewage water that ends up in rivers has also modified the water properties significantly, causing many frog and turtle populations to disappear from those sites (Ramírez-Bautista et al. 2014; Magno-Benítez et al. 2016).

Myths and other cultural factors

Two cultural aspects that contribute to the detriment of Hidalgo's herpetofauna are the lack of understanding regarding the important roles of amphibians and reptiles in ecosystems, and harmful misconceptions that often lead to direct persecution (Cruz-Elizalde et al. 2017). For instance, many people in Hidalgo believe that some species of salamanders (genera *Aquiloerycea* and *Chiropterotriton*) and lizards (genera *Abronia* and *Barisia*) are venomous, while all snakes are indiscriminately regarded as dangerous, and, therefore, killed on sight. Additionally, many people believe that the salamanders *Aquiloerycea cephalica*, *Bolitoglossa platydactyla*, *Chiropterotriton arboreus*, *C. chondrostega*, and the snake *Pituophis deppei* somehow impregnate women; therefore, encounters with these creatures frequently



Fig. 7. Forest fires. A forest fire for land use conversion in the vicinity of El Naranjal, in the municipality of Pisaflores. Photo by Christian Berriozabal-Islas.



Fig. 8. Deforestation for ranching purposes. Cattle pasture in the municipality of Tepehuacan de Guerrero. Photo by Christian Berriozabal-Islas.

end up with them being killed (Ramírez-Bautista et al. 2014; Cruz-Elizalde et al. 2017). Consumption of most herpetofaunal members has not been well-documented in the state. In some rural communities, however, the salamander *Ambystoma velasci* is known to be part of the diet (Fig. 6) and rattlesnakes are used as part of the folk medicine by some inhabitants (Cruz-Elizalde et al. 2017).

Diseases

Globally, many amphibian populations are disappearing due to chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd; Skerrat et al. 2007). Unfortunately, this disease was reported recently in Hidalgo in the anurans *Craugastor rhodopsis*, *Lithobates berlandieri*, *L. johni*, and *Rheohyla miotympanum* (Hernández-Austria 2017). Two factors have been identified as the main drivers of the successful spread of this infection in Hidalgo: the exotic American Bullfrog (*L. catesbeianus*) and global climate change (Kriger et al. 2006). Monitoring of this infection is necessary in order to assess its impact on the diverse native frog populations (Hernández-Austria 2017).

Under these circumstances, government authorities



Fig. 9. Deforestation for agricultural purposes. Change in land use for agricultural purposes in the vicinity of San Cristobal in the municipality of Metztlán. *Photo by Cristian Raúl Olvera-Olvera.*



Fig. 10. Invasive species. *Lithobates catesbeianus* in the vicinity of El Naranjal in the municipality of San Felipe Orizatlán. *Photo by Cristian Raúl Olvera-Olvera.*



Fig. 11. Pet trade. A boa constrictor (*Boa imperator*) captured by locals for maintenance in captivity as a pet in El Borbollon in the municipality of Huehuetla. *Photo by Christian Berriozabal-Islas.*



Fig. 12. Urbanization. Urban growth in the vicinity of Molango de Escamilla in the municipality of the same name. *Photo by Cristian Raúl Olvera-Olvera.*

at all levels and conservation groups must invest more effort in the protection of these species and the habitats where they are found. These efforts are particularly critical in regions that harbor species and habitats that are already vulnerable to anthropogenic stressors. Another critical step is that the respective authorities need to invest more resources in the continuous education of the general public on the importance of the herpetofauna of the state. Otherwise, adequate protection of these species will always remain an elusive goal.

Conservation Status

The conservation status of the members of the herpetofauna of Hidalgo is assessed here using the same three systems of conservation assessment as in the previous entries in the Mexican Conservation Series (see above). These systems are those of SEMARNAT (2010), the IUCN Red List (<http://iucnredlist.org>), and the EVS (Wilson et al. 2013a,b), and these three systems have been updated as necessary.

The SEMARNAT System

The SEMARNAT system is a means of conservation

status assessment developed and implemented by the Secretaría del Medio Ambiente y Recursos Naturales of the federal government of Mexico (SEMARNAT 2010). The ratings are available for some of the herpetofaunal species inhabiting Hidalgo as shown in Table 7 and summarized in Table 9. Three categories of assessment exist in the SEMARNAT system: Endangered (P), Threatened (A), and Under Special Protection (Pr); and the species remaining unassessed in this system are assigned a “No Status” (NS) category.

The data in Table 9 indicate that of the 200 native species in Hidalgo, only 93 (45.8%) have been evaluated using this system. This leaves 107 (52.7%) without a conservation assessment based on SEMARNAT.

If one can assume that SEMARNAT personnel have placed a greater emphasis on species endemic to Mexico or some portion thereof (i.e., a single state), then that consideration should be evident from a comparison of the assignments to both distributional categories and to SEMARNAT categories. In order to determine whether this sort of bias is evident, such comparisons are presented in Table 10. These data demonstrate that the majority of the non-endemic species (61 of 92; 66.3%) currently remain unevaluated in the SEMARNAT system. The comparable figures are 44 of the 104 (42.3%) country



No. 25. *Conopsis lineata* (Kennicott 1859). The Lined Toluca Ground Snake occurs in the central Mexican states of Guanajuato, Guerrero, Jalisco, Estado de México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz, and Ciudad de México (Ramírez-Bautista et al. 2014). This individual was found at Puente de las Cillas, in the municipality of Singuilucan. Wilson (2013a) determined its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status is assessed as Least Concern by the IUCN, but this species is not listed by SEMARNAT. *Photo by Cristian Raúl Olvera-Olvera.*



No. 26. *Lampropeltis annulata*. Kennicott 1861. The Mexican Milksnake is distributed in Nuevo León, Querétaro, and Tamaulipas, and perhaps Coahuila, eastern San Luis Potosí, and Hidalgo (Ruane et al. 2014). This individual was encountered at Venados, in the municipality of Metztitlán. Wilson et al. (2013a) calculated its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has not been evaluated by the IUCN, and this species is not listed by SEMARNAT. *Photo by Cristian Raúl Olvera-Olvera.*



No. 27. *Pituophis deppei* (Duméril 1853). The Mexican Bull Snake occurs in the states of Aguascalientes, Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Nuevo León, Oaxaca, Puebla, San Luis Potosí, Querétaro, Tlaxcala, Veracruz, Zacatecas, and Ciudad de México (Ramírez-Bautista et al. 2014). This individual was encountered in the municipality of Mineral El Chico. Wilson et al. (2013a) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Least Concern by the IUCN, and it is placed in the Threatened (A) category by SEMARNAT. *Photo by Uriel Hernández-Salinas.*



No. 28. *Chersodromus rubriventris* (Taylor 1949). The Redbelly Earth Runner “is found in the Sierra Madre Oriental in the States of San Luis Potosí, Querétaro and Hidalgo” (Canseco-Márquez et al. 2018: 159). This individual was photographed at Chilijapa, in the municipality of Tepehuacan de Guerrero. Wilson et al. (2013a) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Endangered by the IUCN, and it is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

Table 8. Summary of the distributional status of herpetofaunal families in Hidalgo, Mexico.

Family	Number of species	Distributional status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
Bufonidae	6	4	2	—	—
Craugastoridae	5	1	4	—	—
Eleutherodactylidae	5	2	3	—	—
Hylidae	15	5	10	—	—
Leptodactylidae	2	2	—	—	—
Microhylidae	1	1	—	—	—
Ranidae	5	1	3	—	1
Rhinophrynidae	1	1	—	—	—
Scaphiopodidae	2	2	—	—	—
Subtotal	42	19	22	—	1
Ambystomatidae	1	—	1	—	—
Plethodontidae	15	—	11	4	—
Salamandridae	1	1	—	—	—
Subtotal	17	1	12	4	—
Total	59	20	34	4	1
Crocodylidae	1	1	—	—	—
Subtotal	1	1	—	—	—
Anguidae	5	2	3	—	—
Corytophanidae	3	3	—	—	—
Dactyloidae	5	4	1	—	—
Dibamidae	1	—	1	—	—
Eublepharidae	1	1	—	—	—
Gekkonidae	1	—	—	—	1
Iguanidae	1	1	—	—	—
Phrynosomatidae	14	4	10	—	—
Scincidae	2	1	1	—	—
Sphenomorphidae	2	—	2	—	—
Teiidae	2	1	1	—	—
Xantusiidae	4	1	3	—	—
Xenosauridae	3	—	3	—	—
Subtotal	44	18	25	—	1
Boidae	1	1	—	—	—
Colubridae	32	19	13	—	—
Dipsadidae	27	16	11	—	—
Elapidae	2	2	—	—	—
Leptotyphlopidae	3	1	2	—	—
Natricidae	13	6	7	—	—
Sibynophiidae	1	1	—	—	—
Typhlopidae	1	—	—	—	1
Viperidae	13	4	9	—	—
Subtotal	93	50	42	—	1
Emydidae	2	1	1	—	—
Kinosternidae	4	2	2	—	—
Subtotal	6	3	3	—	—
Total	144	72	70	—	2
Sum Total	203	92	104	4	3

endemics and two of the four state endemics (50.0%). Although these figures do not indicate a clear bias in favor of the Mexican endemic species, they do demonstrate that the SEMARNAT system will not be of much use in assessing the conservation status of the Mexican herpetofauna, and specifically the Hidalgo herpetofauna,

until all species are included.

The IUCN System

The IUCN system of conservation assessment is intended to be applicable to all organisms, although most of its evaluations are applied to vertebrate animals and

Table 9. SEMARNAT categorizations for herpetofaunal species in Hidalgo, Mexico, arranged by family. Non-native species are excluded.

Family	Number of species	SEMARNAT categorization			
		Endangered (P)	Threatened (A)	Special protection (Pr)	No status (NS)
Bufonidae	6	—	—	—	6
Craugastoridae	5	—	—	2	3
Eleutherodactylidae	5	—	—	1	4
Hylidae	15	—	4	3	8
Leptodactylidae	2	—	—	—	2
Microhylidae	1	—	—	—	1
Ranidae	4	1	—	2	1
Rhinophrynidae	1	—	—	1	—
Scaphiopodidae	2	—	—	—	2
Subtotal	41	1	4	9	27
Ambystomatidae	1	—	—	1	—
Plethodontidae	15	—	3	9	3
Salamandridae	1	1	—	—	—
Subtotal	17	1	3	10	3
Total	58	2	7	19	30
Crocodylidae	1	—	—	1	—
Subtotal	1	—	—	1	—
Anguidae	5	—	—	3	2
Corytophanidae	3	—	—	2	1
Dactyloidae	5	—	—	1	4
Dibamidae	1	—	1	—	—
Eublepharidae	1	—	1	—	—
Iguanidae	1	—	—	1	—
Phrynosomatidae	14	—	1	2	11
Scincidae	2	—	—	1	1
Sphenomorphidae	2	—	1	1	—
Teiidae	2	—	—	—	2
Xantusiidae	4	—	—	4	—
Xenosauridae	3	—	—	1	2
Subtotal	43	—	4	16	23
Boidae	1	—	—	—	1
Colubridae	32	—	9	2	21
Dipsadidae	27	—	—	11	16
Elapidae	2	—	—	1	1
Leptotyphlopidae	3	—	—	—	3
Natricidae	13	—	8	—	5
Sibynophiidae	1	—	—	—	1
Viperidae	13	—	4	5	4
Subtotal	92	—	21	19	52
Emydidae	2	—	—	—	2
Kinosternidae	4	—	—	4	—
Subtotal	6	—	—	4	2
Total	142	—	25	40	77
Sum Total	200	2	32	59	107

flowering plants. For example, of 67,222 animal species assessed, 46,092 are vertebrates (68.6%); and of 24,230 plant species evaluated, 22,566 (93.1%) are flowering plants (IUCN Red List version 2017-3: Tables 3a,b in that list). The vertebrate animal assessments include 6,609 for amphibians and 6,278 for reptiles (IUCN Red List version 2017-3: Table 3a). The Reptile Database website (<http://www.reptile-database.org/>; accessed 17 April 2018) provides a February 2018 total count for

reptiles of 10,711; thus, 58.6% of the world’s recognized reptile species have been assessed by the IUCN; while the figure for amphibians is 84.4% of 7,832 species (Amphibian Species of the World, <http://research.amnh.org/vz/herpetology/amphibia/>; accessed 17 April 2018). Thus, a significantly greater proportion of amphibian species have been assessed than reptile species. For the global herpetofauna, 12,887 (69.5%) of 18,543 total species have been assessed.

Table 10. Comparison of SEMARNAT and distributional categorizations.

Distributional category	SEMARNAT category				
	Endangered (P)	Threatened (A)	Special Protection (Pr)	No Status (NS)	Total
Non-endemic (NE)	1	9	21	61	92
Country-endemic (CE)	1	23	36	44	104
State-endemic (SE)	—	—	2	2	4
Total	2	32	59	107	200

Table 11. IUCN Red List categorizations for herpetofaunal families in Hidalgo, Mexico. Non-native species are excluded. The shaded columns to the left are the “threatened” categories, and those to the right are the categories which indicate that available conservation status data are too limited to allow the species to be placed in any other IUCN category, or the species has not been evaluated.

Family	Number of species	IUCN Red List categorization						
		Critically Endangered	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated
Bufonidae	6	—	—	—	—	5	—	1
Craugastoridae	5	—	—	2	1	2	—	—
Eleutherodactylidae	5	—	—	2	—	3	—	—
Hylidae	15	1	3	1	2	8	—	—
Leptodactylidae	2	—	—	—	—	2	—	—
Microhylidae	1	—	—	—	—	1	—	—
Ranidae	4	—	1	—	—	3	—	—
Rhinophrynidae	1	—	—	—	—	1	—	—
Scaphiopodidae	2	—	—	—	—	2	—	—
Subtotal	41	1	4	5	3	27	—	1
Ambystomatidae	1	—	—	—	—	1	—	—
Plethodontidae	15	6	4	2	2	—	—	1
Salamandridae	1	—	1	—	—	—	—	—
Subtotal	17	6	5	2	2	1	—	1
Total	58	7	9	7	5	28	—	2
Crocodylidae	1	—	—	—	—	1	—	—
Subtotal	1	—	—	—	—	1	—	—
Anguidae	5	—	—	1	—	4	—	—
Corytophanidae	3	—	—	—	—	3	—	—
Dactyloidae	5	—	—	1	—	—	—	4
Dibamidae	1	—	—	—	—	1	—	—
Eublepharidae	1	—	—	—	—	1	—	—
Iguanidae	1	—	—	—	—	—	—	1
Phrynosomatidae	14	—	—	1	—	12	—	1
Scincidae	2	—	—	—	—	2	—	—
Sphenomorphidae	2	—	—	—	—	2	—	—
Teiidae	2	—	—	—	—	1	—	1
Xantusiidae	4	—	—	1	—	3	—	—
Xenosauridae	3	—	1	—	—	—	—	2
Subtotal	43	—	1	4	—	29	—	9
Boidae	1	—	—	—	—	—	—	1
Colubridae	32	—	1	—	1	24	—	6
Dipsadidae	27	—	2	—	—	13	4	8
Elapidae	2	—	—	—	—	2	—	—
Leptotyphlopidae	3	—	—	—	—	2	—	1
Natricidae	13	—	1	2	—	10	—	—
Sibynophiidae	1	—	—	—	—	1	—	—
Viperidae	13	—	—	—	—	10	—	3
Subtotal	92	—	4	2	1	62	4	19
Emydidae	2	—	—	1	—	—	—	1
Kinosternidae	4	—	—	—	1	2	—	1
Subtotal	6	—	—	1	1	2	—	2
Total	142	—	5	7	2	94	4	30
Sum Total	200	7	14	14	7	122	4	32
Category Total	200	35			129		36	

Table 12. Environmental Vulnerability Scores (EVS) for herpetofaunal species in Hidalgo, Mexico, arranged by family. Shaded area to the left encompasses low vulnerability scores, and the shaded area to the right indicates high vulnerability scores. Non-native species are excluded.

Family	Number of species	Environmental Vulnerability Score																	
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Bufo	6	1	—	1	2	—	—	—	—	2	—	—	—	—	—	—	—	—	
Craugastoridae	5	—	—	—	—	—	1	—	1	—	—	—	2	1	—	—	—	—	
Eleutheroactylidae	5	—	—	—	—	—	—	—	—	1	2	—	—	1	1	—	—	—	
Hylidae	15	1	2	—	—	1	1	2	1	2	—	3	1	—	—	1	—	—	
Leptodactylidae	2	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	
Microhylidae	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Ranidae	4	—	—	—	—	1	—	—	—	—	1	1	1	—	—	—	—	—	
Rhinophrynidae	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	
Scaphiropodidae	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	
Subtotals	41	3	3	2	4	2	3	2	2	5	3	4	4	2	1	1	—	—	
Ambystomatidae	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	
Plethodontidae	15	—	—	—	—	—	—	—	—	—	1	—	1	2	4	3	4	—	
Salamandridae	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	
Subtotal	17	—	—	—	—	—	—	—	1	—	2	—	1	2	4	3	4	—	
Total	58	3	3	2	4	2	3	2	3	5	5	4	5	4	5	4	4	—	
Crocodylidae	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	
Subtotal	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	
Anguidae	5	—	—	—	1	—	—	—	—	—	1	1	1	1	—	—	—	—	
Corytophanidae	3	—	—	—	—	1	1	—	—	—	—	1	—	—	—	—	—	—	
Dactyloidae	5	—	—	—	—	—	2	2	—	—	—	1	—	—	—	—	—	—	
Dibamidae	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	
Eublepharidae	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	
Iguanidae	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	
Phrynosomatidae	14	—	—	1	1	—	—	1	—	1	3	4	2	1	—	—	—	—	
Scincidae	2	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	
Sphenomorphidae	2	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	
Teiidae	2	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	
Xantusiidae	4	—	—	—	—	—	1	—	—	1	—	1	1	—	—	—	—	—	
Xenosauridae	3	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	
Subtotal	43	—	—	1	2	1	4	5	2	4	7	8	4	3	2	—	—	—	
Boidae	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	
Colubridae	32	—	—	2	7	—	2	3	3	2	2	6	2	2	1	—	—	—	
Dipsadidae	27	—	1	1	3	3	2	3	4	—	2	2	3	3	—	—	—	—	
Elapidae	2	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	
Leptotyphlopidae	3	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	
Natricidae	13	—	—	—	—	3	1	—	2	1	—	1	1	4	—	—	—	—	
Sibynophiidae	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	
Viperidae	13	—	—	—	—	—	1	1	—	1	1	1	2	1	3	2	—	—	
Subtotal	92	—	1	3	10	6	7	7	10	6	5	13	8	10	4	2	—	—	
Emydidae	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
Kinosternidae	4	—	—	—	—	—	—	—	2	1	—	—	1	—	—	—	—	—	
Subtotal	6	—	—	—	—	—	—	—	2	1	—	—	1	—	—	—	—	2	
Total	142	—	1	4	12	7	11	12	14	11	12	22	13	13	6	2	—	2	
Sum Total	200	3	4	6	16	9	14	14	17	16	17	26	18	17	11	6	4	2	
Category Total	200	66						76						58					



No. 29. *Imantodes cenchoa* (Linnaeus 1758). The Bluntheaded Tree Snake is broadly distributed “from Tamaulipas and Oaxaca, Mexico, southward through much of Central America to Ecuador, on the Pacific versant, and to Paraguay on the cisandean side of South America” (Lemos-Espinal and Dixon 2013: 191). This individual was found at Cececamel in the municipality of San Felipe Orizatlán. Wilson et al. (2013a) assessed its EVS as 6, placing it in the middle of the low vulnerability category. Its conservation status has not been evaluated at the IUCN, but its SEMARNAT status is judged as Special Protection (Pr). *Photo by Cristian Raúl Olvera-Olvera.*



No. 30. *Micrurus diastema* (Duméril, Bibron, and Duméril 1854). The Variable Coral Snake is found “on the Atlantic versant from northern Veracruz and northern Oaxaca, Mexico, to northwestern Honduras” (McCranie 2011: 457). This individual was photographed at Laguna de Atezca, in the municipality of Molango de Escamilla. Wilson (2013a) calculated its EVS as 8, placing it in the upper portion of the low vulnerability category. Its conservation status has been assessed as Least Concern by the the IUCN, and this elapid is listed as a species of Special Protection (Pr) by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 31. *Metlapilcoatlus nummifer* (Rüppell 1845). The Mexican Jumping Viper is found “from San Luis Potosí southward through Hidalgo and west-central Veracruz to northern and southeastern Oaxaca (Lemos-Espinal and Dixon 2013: 246). This individual was found in El Pinalito, in the municipality of Jacala de Ledezma. Wilson et al. (2013a) estimated its EVS as 13, placing it at the upper limit of the medium vulnerability category. Its conservation status is indicated as Least Concern by the IUCN and as Threatened (A) by SEMARNAT. *Photo by Christian Berriozabal-Islas.*



No. 32. *Bothrops asper* (Garman 1884). The Terciopelo is a wide-ranging pit viper occurring “from southwestern Tamaulipas, Mexico, to coastal Venezuela on the Atlantic versant, and from Costa Rica to southern Ecuador on the Pacific versant, with a disjunct population occurring in southern Chiapas, Mexico, and adjacent Guatemala” (Lemos-Espinal and Dixon 2013: 247). This individual was found in La Esperanza II, in the municipality of Huehuetla. Wilson et al. (2013a) determined its EVS as 12, placing it in the upper portion of the medium vulnerability category. Its conservation status has not been determined by the IUCN, and this species is not listed by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

In previous entries in the Mexican Conservation Series (e.g., Woolrich-Piña et al. 2017), the IUCN system of conservation evaluation has been criticized for several reasons. Nonetheless, the IUCN system is sufficiently broadly applied that its comparison here to the other systems is instructive. Thus, the IUCN categorizations for the members of the Hidalgo herpetofauna are shown in Table 7 and summarized in Table 11.

Of 200 native members of the herpetofauna, 164 (82.0%) have been assessed by the IUCN system. This percentage is similar to that found by Woolrich-Piña et al. (2017) for the herpetofauna of the adjacent state of Puebla (79.5%). Of these 164 species, 35 have been placed in one of the three IUCN “threat categories:” seven as CR, 14 as EN, and 14 as VU (Table 11). The seven CR species are *Bromeliophyla dendroscarta*, *Chiropterotriton arboreus*, *C. chiropterus*, *C. magnipes*, *C. mosaueri*, *C. terrestris*, and *Isthmura gigantea*. Five of these species are country endemics and two are state endemics; one is an anuran and six are salamanders. The 14 EN species are *Sarcohyala arborescendens*, *S. charadricola*, *S. robertorum*, *Lithobates johnei*, *Chiropterotriton chondrostega*, *C. dimidiatus*, *C. multidentatus*, *Pseudoeurycea altamontana*, *Notophthalmus meridionalis*, *Xenosaurus newmanorum*, *Ficimia hardyi*, *Chersodromus rubriventris*, *Rhadinaea marcellae*, and *Thamnophis melanogaster*; and include 12 country endemics, one state endemic and one non-endemic. Four of these species are anurans, five are salamanders, one is a lizard, and four are snakes. The 14 VU species are *Craugastor decoratus*, *C. rhodopsis*, *Eleutherodactylus longipes*, *E. verrucipes*, *Charadrahyla taeniopus*, *Isthmura bellii*, *Pseudoeurycea leprosa*, *Abronia taeniata*, *Norops naufragus*, *Sceloporus megalepidurus*, *Lepidophyma gaigeae*, *Storeria hidalgoensis*, *Thamnophis scaliger*, and *Trachemys venusta*; and include 13 country endemics and one non-endemic. Five of these species are anurans, two are salamanders, four are lizards, two are snakes, and one is a turtle. In total, of the 35 species in the IUCN “threatened categories,” 30 are endemic to Mexico or to Hidalgo (85.7%); 10 species are anurans, 13 are salamanders, five are lizards, six are snakes, and one is a turtle.

Of the 129 species placed in the IUCN “lower risk categories” (NT and LC), only seven (5.4%) are allocated to the NT category; the remaining 122 are placed in the LC category. The seven NT species are *Craugastor berkenbuschii*, *Dryophytes euphorbiaceus*, *Rheohyla miotympanum*, *Aquiloerycea cephalica*, *Bolitoglossa platydactyla*, *Lampropeltis ruthveni*, and *Kinosternon herrerae*. All seven of these species are country endemics; three are anurans, two are salamanders, one is a snake, and one is a turtle.

The 122 LC species comprise 61.0% of the 200 native species in Hidalgo. Whether such a high proportion of these species are actually of “Least Concern” is questionable; and these allocations are examined in detail below.

Thirty-six of the members of the native Hidalgo herpetofauna have not been placed in either the “threatened categories” or the “lower risk categories,” including four allocated to the DD category and 32 to the NE categories. Inasmuch as these 36 species make up 18.0% of the native herpetofauna, they also are examined in greater detail in the following section.

The EVS System

The EVS (Environmental Vulnerability Score) system was developed originally for use in evaluating the conservation status of the Honduran herpetofauna, but has since been deployed in the assessment of other components of the Mexican and Central American herpetofaunas (Wilson et al. 2010, 2013a,b; and all entries in the Mexican Conservation Series [see above]). In the present study, the EVS values for the 200 native species are given in Table 7 and summarized in Table 12.

The EVS values range from 3 to 19, which is one less than the entire theoretical range of 3–20. The most frequent values (applied to 10 or more species) are 6 (16 species), 8 (14), 9 (14), 10 (17), 11 (16), 12 (17), 13 (26), 14 (18), 15 (17), and 16 (11). These ten values are applied to 166 of the 200 native species (83.0%). The lowest score of 3 was calculated for three anuran species (*Rhinella horribilis*, *Smilisca baudinii*, and *Scaphiopus couchii*) and the highest score of 19 was calculated for two turtles (*Terrapene mexicana* and *Trachemys venusta*).

As in prior MCS studies, the EVS are organized here into three categories of low, medium, and high vulnerability. As such, the species counts increase from low vulnerability (66) to medium vulnerability (76), and then decrease in the high vulnerability (58) category. Generally, this pattern is typical of state herpetofaunas that contain more non-endemic species than country and state endemics, as was found in Chiapas (Johnson et al. 2015), Tamaulipas (Terán-Juárez et al. 2016), Nuevo León (Nevárez-de los Reyes et al. 2016), and Coahuila (Lazcano et al. 2019).

When the IUCN categories for the Hidalgo herpetofauna are compared with those from the EVS system (Table 13), 35 of the 58 high vulnerability species (60.3%) are allocated to one of the three IUCN “threat categories.” This relatively high proportion is due primarily to the number of amphibians evaluated by the IUCN as CR, EN, or VU; 23 of 59 amphibian species (39.0%) are anurans (10 species) or salamanders (13), compared to 12 of 144 reptiles (8.3%). No squamates, turtles, or crocodylians are assessed as CR, only five squamates are assessed as EN, and six squamates and one turtle are assessed as VU. At the other extreme, the 66 low vulnerability species (by EVS) comprise 54.1% of the 122 LC species (by IUCN). As demonstrated in previous MCS entries, the results from the IUCN and EVS systems do not complement one another very well.

As reported in previous MCS studies, the main

Table 13. Comparison of Environmental Vulnerability Scores (EVS) and IUCN categorizations for members of the herpetofauna of Hidalgo, Mexico. Non-native species are excluded. Shaded area at the top (EVS scores from 3 to 9) encompasses low vulnerability category scores, and the shaded area at the bottom (EVS scores from 14 to 19) indicates high vulnerability category scores.

IUCN category								
EVS	Critically Endangered	Endangered	Vulnerable	Near Threatened	Least Concern	Data Deficient	Not Evaluated	Total
3	—	—	—	—	2	—	1	3
4	—	—	—	—	4	—	—	4
5	—	—	—	—	4	—	2	6
6	—	—	—	—	11	—	5	16
7	—	—	—	—	8	—	1	9
8	—	—	—	—	11	—	3	14
9	—	—	—	1	10	—	3	14
10	—	—	—	—	15	—	2	17
11	—	1	—	—	13	—	2	16
12	—	2	1	—	10	1	3	17
13	—	2	4	1	17	—	2	26
14	—	3	2	3	7	1	2	18
15	—	3	4	1	6	2	1	17
16	3	—	2	1	3	—	2	11
17	1	3	—	—	1	—	1	6
18	3	—	—	—	—	—	1	4
19	—	—	1	—	—	—	1	2
Total	7	14	14	7	122	4	32	200

reason for the poor correspondence between the IUCN and EVS systems is that a large proportion (158 of 200, 79.0%) of the species are assigned to the NE, DD, and LC categories. Interestingly, the four DD species are all country endemic snakes (*Geophis latifrontalis*, *Hypsiglena tanzeri*, *Rhadinaea gaigeae*, and *R. quinquelineata*). Three of these four species are categorized as high vulnerability species, and the fourth (*R. gaigeae*) has an EVS of 12 putting it in the medium vulnerability category (Table 14). As a result, we believe the conservation needs of these four species are ill-served by leaving them in the DD category of IUCN. Thus, we think that the two species with an EVS of 15 (*Hypsiglena tanzeri* and *Rhadinaea quinquelineata*) would be more appropriately placed in the EN category, the one with an EVS of 14 (*Geophis latifrontalis*) in the VU category, and the one with an EVS of 12 (*Rhadinaea gaigeae*) in the NT category.

Thirty-two of the 200 native species (16.0%) have not been evaluated by the IUCN (Table 15). These 32 species comprise an interesting amalgam of country/state endemics and non-endemic species. Of the 32

species, 11 are species endemic to Hidalgo (one species) or to Mexico (11 species). One of the criticisms levelled against the IUCN system of conservation evaluation is that it is too slow to keep up with taxonomic innovation (Johnson et al. 2015). Of the 11 endemic species listed in Table 15, eight have been described, resurrected from synonymy, or elevated from subspecies to species level in the present decade (i.e., *Chiropterotriton chico*, *Holcosus amphigrammus*, *Xenosaurus mendozai*, *X. tzacualtipantecus*, *Lampropeltis polyzona*, *Geophis lorancai*, *G. turbidus*, *Epictia wynnii*, and *Ophryacus smaragdinus*). Of the 20 non-endemic species not yet assessed by the IUCN, all range into the United States, Central America or both. Clearly, a more rapidly-applied system of conservation assessment is needed, especially given the rate at which anthropogenic habitat modification and destruction occur. As with the DD categorized species, we believe that the EVS provides a means for allocating the NE species to IUCN categories. Thus, we suggest that species with an EVS of 17 or 18 should be placed in the CR category (*Chiropterotriton chico*, *Xenosaurus tzacualtipantecus*, and *Crotalus*

Table 14. Components of the Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Hidalgo, Mexico, that are allocated to the IUCN Data Deficient category. * = country endemic; ** = state endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Geophis latifrontalis</i> *	5	7	2	14
<i>Hypsiglena tanzeri</i> *	5	8	2	15
<i>Rhadinaea gaigeae</i> *	5	5	2	12
<i>Rhadinaea quinquelineata</i> *	5	8	2	15

Table 15. Components of the Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Hidalgo, Mexico, currently classified as Not Evaluated (NE) by the IUCN. Non-native taxa are excluded. * = country endemic; ** = state endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Rhinella horribilis</i>	1	1	1	3
<i>Chiropterotriton chico</i> **	6	8	4	18
<i>Norops laevis</i>	3	3	3	9
<i>Norops lemurinus</i>	3	2	3	8
<i>Norops petersii</i>	2	4	3	9
<i>Norops sericeus</i>	2	3	3	8
<i>Ctenosaura acanthura</i>	2	4	6	12
<i>Sceloporus cyanogenys</i> *	4	6	3	13
<i>Holcosus amphigrammus</i> *	5	3	3	11
<i>Xenosaurus mendozai</i> *	5	8	3	16
<i>Xenosaurus tzacualtipantecus</i> *	6	8	3	17
<i>Boa imperator</i>	3	1	6	10
<i>Drymobius margaritiferus</i>	1	1	4	6
<i>Ficimia olivacea</i> *	5	2	2	9
<i>Lampropeltis annulata</i>	4	3	5	12
<i>Lampropeltis polyzona</i> *	1	3	5	9
<i>Oxybelis aeneus</i>	1	1	3	5
<i>Spilotes pullatus</i>	1	1	4	6
<i>Coniophanes fissidens</i>	1	3	3	7
<i>Geophis lorancai</i> *	5	7	2	14
<i>Geophis turbidus</i> *	5	8	2	15
<i>Hypsiglena jani</i>	1	3	2	6
<i>Imantodes cenchoa</i>	1	3	2	6
<i>Imantodes gemmistratus</i>	1	3	2	6
<i>Leptodeira septentrionalis</i>	2	2	4	8
<i>Sibon nebulatus</i>	1	2	2	5
<i>Epictia wynni</i> *	5	7	1	13
<i>Bothrops asper</i>	3	4	5	12
<i>Crotalus totonacus</i>	5	7	5	17
<i>Ophryacus smaragdinus</i> *	3	6	5	14
<i>Terrapene mexicana</i> *	5	8	6	19
<i>Kinosternon scorpioides</i>	3	4	3	10

totonacus), those with an EVS of 15 or 16 in the EN category (*Xenosaurus mendozai* and *Geophis turbidus*), and those with an EVS of 13 or 14 in the VU category (*Sceloporus cyanogenys*, *Geophis lorancai*, *Epictia wynni*, and *Ophryacus smaragdinus*). The three species with an EVS of 12 perhaps should be allocated to the NT category (*Ctenosaura acanthura*, *Lampropeltis annulata*, and *Bothrops asper*). The remaining species with EVS of 3 to 11 probably should be placed in the LC category.

Previous studies in the MCS series have demonstrated that the largest proportions of the herpetofaunal species found in any of the regions examined were allocated to the LC category by the IUCN. Such is also the case in this study of the Hidalgo herpetofauna. As noted above, 122 of the 200 native species (61.0%) are in this category (Table 16), 52 (42.6%) of which are country endemics. We believe it is unlikely that such a large proportion of these 122 species are really of “Least Concern.” Based on the same reasoning employed with the DD and NE species above, our opinion is that the species with an EVS

of 17 (*Agkistrodon taylori*) should be allocated to the CR category, those with an EVS of 15 or 16 (*Craugastor mexicanus*, *Sceloporus parvus*, *Lampropeltis mexicana*, *Salvadora bairdi*, *Thamnophis pulchrilatus*, *T. sumichrasti*, *Crotalus aquilus*, *C. intermedius*, *C. polystictus*, and *C. triseriatus*) to the EN category, and those with an EVS of 13 or 14 (*Lithobates montezumae*, *L. spectabilis*, *Crocodylus moreletii*, *Barisia imbricata*, *Gerrhonotus infernalis*, *Corytophanes hernandezii*, *Sceloporus aeneus*, *S. bicanthalis*, *S. minor*, *S. mucronatus*, *Lepidophyma occulor*, *Conopsis biserialis*, *C. lineata*, *Leptophis diplotropis*, *Masticophis schotti*, *Pantherophis emoryi*, *Pituophis deppei*, *Trimorphodon tau*, *Geophis mutitorques*, *G. semidoliatus*, *Rena dulcis*, *R. myopica*, *Thamnophis scalaris*, *Metlapilcoatlus nummifer*, and *Crotalus ravus*) to the VU category. Thus, these 31 species comprise 25.4% of the 122 LC species, leaving 91 species that likely should remain in the LC category, at least until more targeted surveys can be undertaken.

Table 16. Components of the Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Hidalgo, Mexico, currently assigned to the IUCN Least Concern (LC) category. Non-native taxa are not included. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Anaxyrus punctatus</i>	1	3	1	5
<i>Incilius marmoreus</i> *	5	5	1	11
<i>Incilius nebulifer</i>	1	4	1	6
<i>Incilius occidentalis</i> *	5	5	1	11
<i>Incilius valliceps</i>	3	2	1	6
<i>Craugastor augusti</i>	2	2	4	8
<i>Craugastor mexicanus</i> *	5	7	4	16
<i>Eleutherodactylus cystignathoides</i>	2	6	4	12
<i>Eleutherodactylus guttifer</i>	2	5	4	11
<i>Eleutherodactylus nitidus</i> *	5	3	4	12
<i>Dryophytes arenicolor</i>	2	4	1	7
<i>Dryophytes eximius</i> *	5	4	1	10
<i>Dryophytes plicatus</i> *	5	5	1	11
<i>Sarcohyala bistincta</i> *	5	3	1	9
<i>Scinax staufferi</i>	2	1	1	4
<i>Smilisca baudinii</i>	1	1	1	3
<i>Tlalocohyla picta</i>	2	5	1	8
<i>Trachycephalus typhonius</i>	1	2	1	4
<i>Leptodactylus fragilis</i>	1	2	2	5
<i>Leptodactylus melanonotus</i>	1	3	2	6
<i>Hypopachus variolosus</i>	2	1	1	4
<i>Lithobates berlandieri</i>	4	2	1	7
<i>Lithobates montezumae</i> *	5	7	1	13
<i>Lithobates spectabilis</i> *	5	6	1	13
<i>Rhinophrynus dorsalis</i>	2	5	1	8
<i>Scaphiopus couchii</i>	1	1	1	3
<i>Spea multiplicata</i>	1	4	1	6
<i>Ambystoma velasci</i> *	5	4	1	10
<i>Crocodylus moreletii</i>	2	5	6	13
<i>Barisia imbricata</i> *	5	6	3	14
<i>Gerrhonotus infernalis</i>	5	5	3	13
<i>Gerrhonotus liocephalus</i>	2	1	3	6
<i>Gerrhonotus ophiurus</i> *	5	4	3	12
<i>Basiliscus vittatus</i>	1	3	3	7
<i>Corytophanes hernandezii</i>	4	6	3	13
<i>Laemactis serratus</i>	2	3	3	8
<i>Anelytropsis papillosus</i> *	5	4	1	10
<i>Coleonyx elegans</i>	2	3	4	9
<i>Phrynosoma orbiculare</i> *	5	4	3	12
<i>Sceloporus aeneus</i> *	5	5	3	13
<i>Sceloporus bicanthalis</i> *	5	5	3	13
<i>Sceloporus grammicus</i>	2	4	3	9
<i>Sceloporus minor</i> *	5	6	3	14
<i>Sceloporus mucronatus</i> *	5	5	3	13
<i>Sceloporus parvus</i> *	5	7	3	15
<i>Sceloporus scalaris</i> *	5	4	3	12
<i>Sceloporus serrifer</i>	2	1	3	6
<i>Sceloporus spinosus</i> *	5	4	3	12
<i>Sceloporus torquatus</i> *	5	3	3	11
<i>Sceloporus variabilis</i>	1	1	3	5
<i>Plestiodon lynx</i> *	5	2	3	10
<i>Plestiodon tetragrammus</i>	4	5	3	12
<i>Scincella gemmingeri</i> *	5	3	3	11
<i>Scincella silvicola</i> *	5	4	3	12
<i>Aspidoscelis gularis</i>	2	4	3	9
<i>Lepidophyma flavimaculatum</i>	1	5	2	8
<i>Lepidophyma occulor</i> *	5	7	2	14
<i>Lepidophyma sylvaticum</i> *	5	4	2	11
<i>Coluber constrictor</i>	1	6	3	10
<i>Conopsis biserialis</i> *	5	6	2	13

Table 16 (continued). Components of the Environmental Vulnerability Scores (EVS) for members of the herpetofauna of Hidalgo, Mexico, currently assigned to the IUCN Least Concern (LC) category. Non-native taxa are not included. * = country endemic.

Species	Environmental Vulnerability Score (EVS)			
	Geographic Distribution	Ecological Distribution	Reproductive Mode/Degree of Persecution	Total Score
<i>Conopsis lineata</i> *	5	6	2	13
<i>Conopsis nasus</i> *	5	4	2	11
<i>Drymarchon melanurus</i>	1	1	4	6
<i>Drymobius chloroticus</i>	1	3	4	8
<i>Ficimia streckeri</i> *	3	7	2	12
<i>Lampropeltis mexicana</i> *	5	7	3	15
<i>Leptophis diplotropis</i> *	5	5	4	14
<i>Leptophis mexicanus</i>	1	1	4	6
<i>Masticophis flagellum</i>	1	3	4	8
<i>Masticophis mentovarious</i>	1	1	4	6
<i>Masticophis schotti</i>	4	5	4	13
<i>Mastigodryas melanolomus</i>	1	1	4	6
<i>Pantherophis emoryi</i>	3	6	4	13
<i>Pituophis catenifer</i>	4	1	4	9
<i>Pituophis deppei</i> *	5	5	4	14
<i>Pseudelaphe flavirufa</i>	2	4	4	10
<i>Salvadora bairdi</i> *	5	6	4	15
<i>Salvadora grahamiae</i>	4	2	4	10
<i>Senticolis triaspis</i>	2	1	3	6
<i>Tantilla bocourti</i> *	5	2	2	9
<i>Tantilla rubra</i>	2	1	2	5
<i>Trimorphodon tau</i> *	5	4	4	13
<i>Adelphicos quadrivirgatum</i>	4	4	2	10
<i>Amastridium sapperi</i>	4	4	2	10
<i>Coniophanes imperialis</i>	2	3	3	8
<i>Coniophanes piceivittis</i>	1	3	3	7
<i>Diadophis punctatus</i>	1	1	2	4
<i>Geophis mutitorques</i> *	5	6	2	13
<i>Geophis semidoliatus</i> *	5	6	2	13
<i>Leptodeira maculata</i>	2	1	4	7
<i>Ninia diademata</i>	4	3	2	9
<i>Pliocercus elapoides</i>	4	1	5	10
<i>Rhadinaea decorata</i>	1	6	2	9
<i>Rhadinaea hesperia</i>	5	3	2	10
<i>Tropidodipsas sartorii</i>	2	2	5	9
<i>Micrurus diastema</i>	2	1	5	8
<i>Micrurus tener</i>	1	5	5	11
<i>Rena dulcis</i>	4	8	1	13
<i>Rena myopica</i> *	5	7	1	13
<i>Nerodia rhombifer</i>	1	5	4	10
<i>Storeria dekayi</i>	1	4	2	7
<i>Storeria storerioides</i> *	5	4	2	11
<i>Thamnophis cyrtopsis</i>	2	1	4	7
<i>Thamnophis eques</i>	2	2	4	8
<i>Thamnophis marcianus</i>	1	5	4	10
<i>Thamnophis proximus</i>	1	2	4	7
<i>Thamnophis pulchrilatus</i> *	5	6	4	15
<i>Thamnophis scalaris</i> *	5	5	4	14
<i>Thamnophis sumichrasti</i> *	5	6	4	15
<i>Scaphiodontophis annulatus</i>	1	5	5	11
<i>Agkistrodon taylori</i> *	5	7	5	17
<i>Crotalus aquilus</i> *	5	6	5	16
<i>Crotalus atrox</i>	1	3	5	9
<i>Crotalus intermedius</i> *	5	5	5	15
<i>Crotalus molossus</i>	2	1	5	8
<i>Crotalus polystictus</i> *	5	6	5	16
<i>Crotalus ravus</i> *	5	4	5	14
<i>Crotalus scutulatus</i>	2	4	5	11
<i>Crotalus triseriatus</i> *	5	6	5	16
<i>Metlapilcoatlus nummifer</i> *	5	3	5	13
<i>Kinosternon hirtipes</i>	2	5	3	10
<i>Kinosternon integrum</i> *	5	3	3	11

Table 17. Number of herpetofaunal species in the four distributional status categories among the four physiographic regions of Hidalgo, Mexico. Rank Order is determined by adding the numbers of Country Endemics and State Endemics.

Physiographic Region	Distributional Status Category				Total	Rank Order
	Non-endemics	Country Endemics	State Endemics	Non-natives		
Sierra Madre Oriental	74	90	1	1	166	1
Trans-Mexican Volcanic Belt	20	59	4	2	84	2
Mexican Plateau	27	49	2	1	79	3
Gulf Coastal Lowlands	60	31	—	2	93	4

Relative Herpetofaunal Priority

Johnson et al. (2015a) developed the concept of Relative Herpetofaunal Priority (RHP), a simple metric used to measure the relative importance of the herpetofaunal species found in any geographic entity (e.g., a state or physiographic region). Determining the RHP involves the use of two methods: (1) calculation of the proportion of state and country endemics as related to the entire physiographic regional herpetofauna, and (2) computation of the absolute number of high EVS category species in each physiographic regional herpetofauna. The pertinent data for these two methods are shown in Tables 17 and 18.

Based on the relative number of country and state endemic species in each physiographic region and the rank the regions occupy, the SMO region occupies rank 1, with 91 endemics out of a total of 166 species (54.8%, Table 17). The other ranks are as follows: second = TMV (63 of 84; 75.0%); third = MXP (51 of 79; 64.6%); and fourth = GCL (31 of 93; 33.3%).

The data in Table 18 indicate that the rank ordering of the four physiographic regions is the same as that documented in Table 17. Based on the relative numbers of high vulnerability species, the SMO region again holds rank 1, with 47 high vulnerability species of a total of 166 species (28.3%). The other ranks are as follows: second = TMV (29 of 82; 35.4%); third = MXP (23 of 77; 29.9%); and fourth = GCL (15 of 91; 16.5%).

Based on the RHP analysis (Tables 17 and 18), the most important physiographic region from a conservation standpoint is clearly the SMO, because it harbors by far the largest number of country and state endemics and the greatest number of high vulnerability species. The 91 endemic species comprise 20 anurans (all country endemics), 11 salamanders (eight country endemics and three state endemics), 57 squamates (all country endemics), and three turtles (all country endemics). These 91 species are indicated in Table 4 with either

single or double asterisks. The SMO also contains 47 high vulnerability species, including seven anurans, nine salamanders, 29 squamates, and two turtles. These 47 species and their respective EVS values are as follows:

*Craugastor decoratus** (15)
*Craugastor rhodopis** (14)
*Eleutherodactylus longipes** (15)
*Eleutherodactylus verrucipes** (16)
*Bromeliophyla dendroscarta** (17)
*Sarcohyala charadricola** (14)
*Lithobates johni** (14)
*Aquiloerycea cephalica** (14)
*Chiropterotriton arboreus** (18)
*Chiropterotriton chondrostega** (17)
*Chiropterotriton dimidiatus** (17)
*Chiropterotriton mosaueri*** (18)
*Chiropterotriton multidentatus** (15)
*Chiropterotriton terrestris** (18)
*Isthmura gigantea** (16)
*Pseudoeurycea leprosa** (16)
*Abronia taeniata** (15)
*Barisia imbricata** (14)
*Sceloporus megalepidurus** (14)
*Sceloporus minor** (14)
*Sceloporus parvus** (15)
*Lepidophyma occulor** (14)
*Xenosaurus mendozai** (16)
*Xenosaurus newmanorum** (15)
*Xenosaurus tzacualtipantecus** (16)
*Lampropeltis mexicana** (15)
*Pituophis deppei** (14)
*Salvadora bairdi** (15)
*Chersodromus rubriventris** (14)
*Geophis latifrontalis** (14)
*Geophis lorancai** (14)
*Geophis turbidus** (15)
*Hypsiglena tanzeri** (15)
*Rhadinaea quinquelineata** (15)

Table 18. Number of herpetofaunal species in the three EVS categories among the four physiographic regions of Hidalgo, Mexico. Rank Order is determined by the relative number of High EVS species. Non-native species are excluded.

Physiographic Region	Low EVS	Medium EVS	High EVS	Total	Rank Order
Sierra Madre Oriental	58	60	47	165	1
Trans-Mexican Volcanic Belt	17	36	29	82	2
Mexican Plateau	21	33	23	77	3
Gulf Coastal Lowlands	43	33	15	91	4

*Thamnophis melanogaster** (15)
*Thamnophis scalaris** (14)
*Thamnophis sumichrasti** (15)
*Agkistrodon taylori** (17)
*Crotalus aquilus** (16)
*Crotalus intermedius** (15)
*Crotalus polystictus** (16)
*Crotalus ravus** (14)
*Crotalus totonacus** (17)
*Crotalus triseriatus** (16)
*Ophryacus smaragdinus** (14)
*Terrapene mexicana** (19)
*Kinosternon herrerae** (14)

Of these 47 species, 46 are country endemics and one is a state endemic; their EVS values range from 14 to 19.

The TMV region occupies the second RHP rank, with 63 country and state endemics (Table 17), including 12 anurans (all country endemics), 14 salamanders (10 country endemics and four state endemics), 36 squamates (all country endemics), and one turtle (a state endemic; Table 4). This region also harbors 29 high vulnerability species (Table 18), including the following two anurans, 12 salamanders, and 15 squamates:

*Eleutherodactylus longipes** (15)
*Eleutherodactylus verrucipes** (16)
*Aquiloerycea cephalica** (14)
*Chiropterotriton arboreus** (18)
*Chiropterotriton chico*** (18)
*Chiropterotriton chiropterus** (16)
*Chiropterotriton chondrostega** (17)
*Chiropterotriton dimidiatus** (17)
*Chiropterotriton magnipes** (16)
*Chiropterotriton mosaueri*** (18)
*Chiropterotriton multidentatus** (15)
*Chiropterotriton terrestris** (18)
*Pseudoeurycea altamontana** (17)
*Pseudoeurycea leprosa** (16)
*Abronia taeniata** (15)
*Barisia imbricata** (14)
*Sceloporus megalepidurus** (14)
*Sceloporus minor** (14)
*Lampropeltis ruthveni** (16)
*Pituophis deppei** (14)
*Rhadinaea quinquelineata** (15)
*Thamnophis pulchrilatus** (15)
*Thamnophis scalaris** (14)
*Thamnophis scaliger** (15)
*Crotalus aquilus** (16)
*Crotalus intermedius** (15)
*Crotalus polystictus** (16)
*Crotalus ravus** (14)
*Crotalus triseriatus** (16)

Twenty-seven of these species are country endemics and the other two are state endemics, and their EVS vary from 14 to 18.

The Mexican Plateau occupies rank three, with 51 country and state endemic species (Table 17), including 10 anurans (all country endemics), seven salamanders (five country endemics and two state endemics), 33 squamates (all country endemics), and one turtle (a country endemic; Table 4). The region also contains 23 high vulnerability species (Table 18), including the following one anuran, five salamanders, and 17 squamates:

*Sarcohya charadricola** (14)
*Chiropterotriton chiropterus** (16)
*Chiropterotriton dimidiatus*** (17)
*Chiropterotriton mosaueri*** (18)
*Chiropterotriton multidentatus** (15)
*Pseudoeurycea leprosa** (16)
*Abronia taeniata** (15)
*Barisia imbricata** (14)
*Sceloporus megalepidurus** (14)
*Sceloporus minor** (14)
*Sceloporus parvus** (15)
*Lampropeltis mexicana** (15)
*Lampropeltis ruthveni** (16)
*Pituophis deppei** (14)
*Salvadora bairdi** (15)
*Rhadinaea quinquelineata** (15)
*Thamnophis melanogaster** (15)
*Thamnophis scaliger** (15)
*Thamnophis sumichrasti** (15)
*Crotalus aquilus** (16)
*Crotalus polystictus** (16)
*Crotalus ravus** (14)
*Crotalus triseriatus** (16)

Twenty-one of these species are country endemics and the other two are state endemics, and their EVS vary from 14 to 18.

The region occupying the fourth rank is the Gulf Coastal Lowlands, which contains 31 country endemic species (Table 17), including eight anurans (all country endemics), one salamander (a country endemic), 22 squamates (all country endemics), and one turtle (a country endemic; Table 4). This region also harbors 15 high vulnerability species (Table 18), including the following five anurans, one salamander, seven squamates, and two turtles:

*Craugastor berkenbuschii** (14)
*Craugastor decoratus** (15)
*Craugastor rhodopis** (14)
*Bromelohyla dendroscarta** (17)
*Lithobates johnei** (14)
*Bolitoglossa platydactyla** (15)
*Lepidophyma occulor** (14)
*Leptophis diplotropis** (14)
*Chersodromus rubriventris** (14)
*Rhadinaea quinquelineata** (15)
*Thamnophis pulchrilatus** (15)
*Agkistrodon taylori** (17)



No. 33. *Crotalus aquilus* Klauber 1952. The Dusky Rattlesnake is found “from the region of Lake Chapala, Jalisco, eastward through Michoacán, Guanajuato, Querétaro, central San Luis Potosí, and southeastward through northern Hidalgo and northwestern Veracruz” (Lemos-Espinal and Dixon 2013: 249). This individual was encountered near Nopalillo, in the municipality of Singuilcan. Wilson (2013a) ascertained its EVS as 16, placing it in the middle portion of the high vulnerability category. It is allocated to the Least Concern category by the IUCN, and is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Cristian Raúl Olvera-Olvera.*



No. 34. *Crotalus atrox* Baird and Girard 1853. The Western Diamond-backed Rattlesnake is broadly distributed in the United States and in Mexico. “In the United States, the distribution...extends from Arkansas and north-central Oklahoma westward to southeastern California and southward through parts of Arizona, New Mexico, and much of Texas. In Mexico, this species ranges from northeastern Baja California through Sonora and northern Sinaloa, across most of Chihuahua except for the Sierra Madre Occidental, throughout Coahuila, Nuevo León, and Tamaulipas, and in the northeastern parts of Durango and Zacatecas. It also occurs in Hidalgo and Querétaro, and in parts of central and eastern San Luis Potosí, as well as in extreme northern Veracruz” (Lemos-Espinal and Dixon 2013: 250). This individual was found at Rancho Alegre, in the municipality of San Agustín Metzquitlán. Wilson et al. (2013a) calculated its EVS as 9, placing it at the upper limit of the low vulnerability category. Its conservation status has been evaluated as Least Concern by the IUCN, and it is allocated to the Special Protection (Pr) category by SEMARNAT. *Photo by Cristian Raúl Olvera-Olvera.*



No. 35. *Crotalus intermedius* Troschel 1865. The Mexican Small-headed Rattlesnake is distributed in “several disjunct populations...in the central and southern highland region of Mexico” (Campbell and Lamar 2004: 553). This individual was found in El Encinal in the municipality of Singuilcan. Wilson et al. (2013b) calculated its EVS as 15, placing it in the lower portion of the high vulnerability category, the IUCN has assessed it as Least Concern, and SEMARNAT listed this rattlesnake as Threatened (A). *Photo by Ferdinand Torres-Angeles.*



No. 36. *Crotalus ravus* Cope 1865. The Mexican Pygmy Rattlesnake is distributed in “temperate montane regions of south-central Mexico” (Heimes 2016: 463). This individual was found at Cerro Hihuingo in the municipality of Tepeapulco. Wilson et al. (2013b) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category, the IUCN has evaluated it as Least Concern, and SEMARNAT lists this rattlesnake as Threatened (A). *Photo by Christian Berriozabal-Islas.*

Table 19. Characteristics of Natural Protected Areas in Hidalgo, Mexico. Abbreviations for **Facilities available** are as follows: A = administrative services; R = park guards; S = systems of pathways; and V = facilities for visitors.

Name	Category	Date of Decree (dd/mm/yyyy)	Area (ha)	Municipalities	Jurisdiction	Physiographic region	Facilities available	Occupied by landowners	Management plan available	Herpetofaunal survey completed
Barranca de Metztitlán	Reserva de la Biosfera	27-11-2000	96,042.9	Acatlán, Atotonilco El Grande, Eloxochitlán, Huasca de Ocampo, Meztlán, San Agustín Metzquititlán, Metepec, Zacualtípán de Ángeles, El Cardonal	Mexican Federal Government	Trans-Mexican Volcanic Belt	A, R	Yes	Yes	Yes
Cuenca Hidrográfica del Río Necaxa	APRN	20-10-1938	42,129.4	Acaxochitlán, Cuauhtemoc de Hinojosa	Mexican Federal Government	Gulf Coastal Lowlands	A, S, V	Yes	Yes	No
El Chico	PN	06-07-1982	2,739.0	Mineral del Chico, Mineral del Monte y Pachuca de Soto	Mexican Federal Government	Transmexican Volcanic Belt	A, R, S, V	Yes	Yes	Yes
Los Mármoles	PN	08-09-1936	23,150.0	Pacula, Jacala de Ledezma, Zimapán y Nicolás Flores	Mexican Federal Government	Transmexican Volcanic Belt	A, R, V	Yes	Yes	No
Tula	PN	27-05-1981	99.5	Tula de Allende	Mexican Federal Government	Transmexican Volcanic Belt	A, R, S, V	Yes	No	No

*Crotalus totonacus** (17)
Trachemys venusta (19)
*Kinosternon herrerae** (14)

Fourteen of these species are country endemics and the other one is a non-endemic, and their EVS range from 14 to 19.

Fifty-eight members of the Hidalgo herpetofauna are allocated to the high vulnerability category (Table 12), thus the proportion of these species recorded in the four physiographic regions are as follows: SMO (81.0%); TMV (52.7%); MXP (41.8%); and GCL (27.3%). These data should figure prominently in conservation management plans for the state.

Protected Areas in Hidalgo

Generally, the purpose of natural protected areas is to allow for the continued functioning of ecosystem services that are dependent on the interactions among the components of the atmosphere, hydrosphere, lithosphere, and biosphere. Thus, the protected areas that do the best job of guarding ecosystem services are those left in a state that is as close to a natural state as possible (Ervin 2003; Gaston et al. 2008). Unfortunately, in a world overrun by populations of the principal invasive species, *Homo sapiens*, areas can be maintained in a natural state only if they are overseen by professional conservation managers who are assigned to legally constituted protected areas.

In an effort to assess the state of Hidalgo’s protected areas, a variety of data on these areas was assembled (Table 19). The number of these protected areas in Hidalgo is relatively small (five) compared to, for example, the 14 found in the adjacent state of Puebla (Woolrich-Piña et al. 2017). These five areas are all administered by the Mexican federal government and include a biosphere reserve, an Area of Protection of Natural Resources, and three national parks (Table 19). The five areas range in size from 99.5 to 96,042.9 ha. The total area is 164,160.8 ha or 1,641.6 km², which is 7.9% of the area of the state (20,813 km²; <http://cuentame.inegi.org.mx/monografias/informacion/hgo/>). They were established during the period of 1936 to 2000.

The representation of these areas among the four physiographic regions is skewed heavily toward the Trans-Mexican Volcanic Belt; inasmuch as four of the five areas are located in this region, while the other area is found within the Gulf Coastal Lowlands. Thus, there is no representation within the Mexican Plateau or the Sierra Madre Oriental. This fact has major consequences for the protection of the herpetofauna of Hidalgo, especially since the Sierra Madre Oriental is shown above to be the most significant region in Hidalgo for the herpetofauna, due to the presence of high numbers of endemic and high vulnerability species.

Considering the range of facilities available in these protected areas, only two of them have a full range (as

Table 20. Distribution of herpetofaunal species in Natural Protected Areas of Hidalgo, Mexico, based on herpetofaunal surveys. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Natural Protected Areas				
	Barranca de Metztitlán	Cuenca Hidrográfica del Río Necaxa	El Chico	Los Mármoles	Tula
Anura (15 species)					
Bufonidae (3 species)					
<i>Incilius occidentalis</i> *		+			
<i>Incilius valliceps</i>	+			+	
<i>Rhinella horribilis</i>	+			+	
Craugastoridae (2 species)					
<i>Craugastor augusti</i>	+			+	
<i>Craugastor rhodopsis</i> *		+			
Eleutherodactylidae (1 species)					
<i>Eleutherodactylus verrucipes</i> *				+	
Hylidae (6 species)					
<i>Charadrahyla taeniopus</i> *		+			
<i>Dryophytes arenicolor</i>		+		+	
<i>Dryophytes euphorbiaceus</i> *		+			
<i>Dryophytes eximius</i> *	+	+	+	+	
<i>Dryophytes plicatus</i> *			+	+	
<i>Rheohyla miotympanum</i> *	+	+		+	
Ranidae (2 species)					
<i>Lithobates berlandieri</i>	+			+	+
<i>Lithobates spectabilis</i> *		+	+	+	+
Scaphiopodidae (1 species)					
<i>Spea multiplicata</i>	+			+	
Caudata (8 species)					
Ambystomatidae (1 species)					
<i>Ambystoma velasci</i> *		+	+		
Plethodontidae (7 species)					
<i>Aquiloerycea cephalica</i> *			+	+	
<i>Chiropterotriton chondrostega</i> *		+		+	
<i>Chiropterotriton dimidiatus</i> **			+		
<i>Chiropterotriton mosaueri</i> **				+	
<i>Chiropterotriton multidentatus</i> *			+		
<i>Isthmura bellii</i> *				+	
<i>Pseudoeurycea altamontana</i> *			+		
Squamata (54 species)					
Anguidae (5 species)					
<i>Abronia taeniata</i> *			+	+	
<i>Barisia imbricata</i> *		+	+	+	+
<i>Gerrhonotus infernalis</i>	+				
<i>Gerrhonotus liocephalus</i>					+

Table 20 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Hidalgo, Mexico, based on herpetofaunal surveys. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Natural Protected Areas				
	Barranca de Metztitlán	Cuenca Hidrográfica del Río Necaxa	El Chico	Los Mármoles	Tula
<i>Gerrhonotus ophiurus</i> *				+	
Phrynosomatidae (10 species)					
<i>Phrynosoma orbiculare</i> *	+	+	+	+	+
<i>Sceloporus aeneus</i> *		+			
<i>Sceloporus bicanthalis</i> *			+		
<i>Sceloporus grammicus</i>	+	+	+	+	+
<i>Sceloporus minor</i> *	+			+	
<i>Sceloporus mucronatus</i> *	+	+	+		+
<i>Sceloporus parvus</i> *	+			+	
<i>Sceloporus spinosus</i> *	+		+	+	+
<i>Sceloporus torquatus</i> *	+			+	
<i>Sceloporus variabilis</i>	+	+		+	
Scincidae (1 species)					
<i>Plestiodon lynxe</i> *	+	+	+	+	
Sphenomorphidae (1 species)					
<i>Scincella gemmingeri</i> *	+	+			
Teiidae (1 species)					
<i>Aspidoscelis gularis</i>	+			+	
Xantusiidae (2 species)					
<i>Lepidophyma gaigeae</i> *				+	
<i>Lepidophyma occulor</i> *	+				
Colubridae (10 species)					
<i>Conopsis biserialis</i> *					
<i>Conopsis lineata</i> *	+		+	+	+
<i>Drymarchon melanurus</i>	+				
<i>Ficimia hardyi</i> *				+	
<i>Masticophis schotti</i>				+	
<i>Pantherophis emoryi</i>				+	
<i>Pituophis deppei</i> *		+	+	+	+
<i>Salvadora bairdi</i> *				+	+
<i>Senticolis triaspis</i>	+				
<i>Trimorphodon tau</i> *	+				
Dipsadidae (7 species)					
<i>Coniophanes fissidens</i>		+			
<i>Diadophis punctatus</i>		+		+	+
<i>Geophis mutitorques</i> *		+		+	
<i>Geophis semidoliatus</i> *	+		+	+	
<i>Hypsiglena tanzeri</i> *	+				

Table 20 (continued). Distribution of herpetofaunal species in Natural Protected Areas of Hidalgo, Mexico, based on herpetofaunal surveys. * = species endemic to Mexico; ** = species endemic to Hidalgo; and *** = non-native species.

Taxa	Natural Protected Areas				
	Barranca de Metztitlán	Cuenca Hidrográfica del Río Necaxa	El Chico	Los Mármoles	Tula
<i>Leptodeira septentrionalis</i>	+				
<i>Rhadinaea gaigeae</i> *			+	+	
Elapidae (1 species)					
<i>Micrurus tener</i>	+				
Leptotyphlopidae (1 species)					
<i>Rena myopica</i> *	+				
Natricidae (10 species)					
<i>Nerodia rhombifer</i>	+				
<i>Storeria hidalgoensis</i> *				+	
<i>Storeria storerioides</i> *		+			
<i>Thamnophis cyrtopsis</i>	+				
<i>Thamnophis eques</i>		+			
<i>Thamnophis proximus</i>	+				
<i>Thamnophis pulchrilatus</i> *			+		
<i>Thamnophis scalaris</i> *		+			
<i>Thamnophis scaliger</i> *		+			
<i>Thamnophis sumichrasti</i> *		+			
Viperidae (5 species)					
<i>Crotalus aquilus</i> *			+	+	+
<i>Crotalus atrox</i>	+			+	
<i>Crotalus molossus</i>	+			+	
<i>Crotalus triseriatus</i> *	+	+		+	
<i>Ophryacus smaragdinus</i> *		+			
Testudines (1 species)					
Kinosternidae (1 species)					
<i>Kinosternon integrum</i> *				+	
Total (78 species)	35	29	22	44	13

indicated in Table 19). A major problem with all of these five areas is that some amount of each is occupied by landowners. Fortunately, however, management plans are available for four of the five areas.

Herpetofaunal surveys have been completed for only two of the five areas, so obviously surveys need to be completed for the remaining three. Even though surveys are incomplete for the protected areas in Hidalgo, the available information on the distribution status of species known to occur in each of these areas have been collated, and are shown in Table 20 and summarized in Table 21.

Of the 200 native species that make up the herpetofauna of Hidalgo, only 78 (39.2%) have been recorded from the five areas combined. The numbers of species recorded from these areas range from 13 in

Parque Nacional Tula to 44 in Parque Nacional Los Mármoles. Of the 103 country endemic species known from Hidalgo, 51 (49.5%) are found in the five areas combined. Of the 92 non-endemic species in the country, only 25 (27.2%) are recorded for these areas. Two of the four state endemic species (50.0%) are documented for only two of the five areas (Parques Nacionales El Chico and Los Mármoles). Finally, on the positive side, none of the three non-native species has been recorded in any of the protected areas. These data demonstrate that completion of herpetofaunal surveys will be a major step toward assessing the conservation needs of the Hidalgo herpetofauna.

Of the 122 native species not found in any of the five protected areas, 53 are country endemics, two are

Table 21. Summary of the distributional status of herpetofaunal species in Protected Areas in Hidalgo, Mexico. Total = total number of species recorded in all of the listed protected areas.

Protected area	Number of species	Distributional status			
		Non-endemic (NE)	Country Endemic (CE)	State Endemic (SE)	Non-native (NN)
RB Barranca de Metztitlán	35	18	17	—	—
APRN Cuenca Hidrográfica del Río Necaxa	29	6	23	—	—
PN El Chico	22	1	21	—	—
PN Los Mármoles	44	14	29	1	—
PN Tula	13	3	10	—	—
Total	78	25	51	2	—

state endemics, and 67 are non-endemics. The 53 country endemics not recorded in any of the protected areas are:

Incilius marmoreus
Craugastor berkenbuschii
Craugastor decoratus
Craugastor mexicanus
Eleutherodactylus longipes
Eleutherodactylus nitidus
Bromeliohyla dendroscarta
Sarcohyla arborescandens
Sarcohyla bistincta
Sarcohyla charadricola
Sarcohyla robertsororum
Lithobates johnei
Lithobates montezumae
Bolitoglossa platydactyla
Chiropterotriton arboreus
Chiropterotriton chiropterus
Chiropterotriton magnipes
Isthmura gigantea
Pseudoeurycea leprosa
Norops naufragus
Anelytropsis papillosus
Sceloporus megalepidurus
Sceloporus scalaris
Scincella silvicola
Holcosus amphigrammus
Lepidophyma sylvaticum
Xenosaurus mendozai
Xenosaurus newmanorum
Xenosaurus tzacualtipantecus
Conopsis nasus
Ficimia olivacea
Lampropeltis mexicana
Lampropeltis polyzona
Lampropeltis ruthveni
Leptophis diplotropis
Tantilla bocourti
Chersodromus rubriventris
Geophis latifrontalis
Geophis lorancai
Geophis turbidus
Rhadinaea hesperia
Rhadinaea marcellae

Rhadinaea quinquelineata
Epictia wynnii
Thamnophis melanogaster
Agkistrodon taylori
Crotalus intermedius
Crotalus polystictus
Crotalus ravus
Crotalus totonacus
Metlapilcoatlus nummifer
Terrapene mexicana
Kinosternon herrerae

The two unrecorded state endemics are:

Chiropterotriton chico
Chiropterotriton terrestris

The 67 non-endemics are:

Anaxyrus punctatus
Incilius nebulifer
Eleutherodactylus cystignathoides
Eleutherodactylus guttilatus
Scinax staufferi
Smilisca baudinii
Tlalocohyla picta
Trachycephalus vermiculatus
Leptodactylus fragilis
Leptodactylus melanonotus
Hypopachus variolosus
Rhinophrynus dorsalis
Scaphiopus couchii
Notophthalmus meridionalis
Crocodylus moreletii
Basiliscus vittatus
Corytophanes hernandezii
Laemactus serratus
Norops laeviventris
Norops lemurinus
Norops petersii
Norops sericeus
Coleonyx elegans
Ctenosaura acanthura
Sceloporus cyanogenys
Sceloporus serrifer



No. 37. *Crotalus scutulatus* Kennicott 1861. The Mohave Rattlesnake occurs “from the Mohave Desert to northern Sonora, and from extreme southern New Mexico and the Big Bend region of Texas southward across the Mexican Plateau to its southern edge” (Heimes 2016: 467). This individual was found San José Atlán, in the municipality of Nopala. Wilson et al. (2013b) calculated its EVS as 11, placing it in the lower portion of the medium vulnerability category, the IUCN assessed it as Least Concern, and SEMARNAT lists this rattlesnake under the category of Special Protection (Pr). *Photo by Christian Berriozabal-Islas.*



No. 38. *Crotalus triseriatus* (Wagler 1830). The Central Plateau Dusky Rattlesnake is distributed in Aguascalientes, Ciudad de México, Durango, Estado de México, Guanajuato, Guerrero, Hidalgo, Michoacán, Morelos, Nayarit, Puebla, Querétaro, San Luis Potosí, Tamaulipas, Tlaxcala, Veracruz, and Zacatecas (Ramírez-Bautista et al. 2014). This individual was secured at Los Reyes, in the municipality of Acaxochitlan. Wilson et al. (2013a) estimated its EVS as 16, placing it in the middle portion of the high vulnerability category. Its conservation status is evaluated as Least Concern by the IUCN, but this species is not listed by SEMARNAT. *Photo by Ferdinand Torres-Angeles.*



No. 39. *Ophryacus smaragdinus* Grünwald, Jones, Franz-Chávez, and Ahumada-Carillo 2015. The Emerald Horned Viper is known from “east-central Hidalgo, west-central Veracruz, northeastern Puebla, and north-central Oaxaca” (Grünwald et al. 2015: 398). This individual was located at Santa Catarina, in the municipality of Tenango de Doria. Woolrich et al. (2017) indicated its EVS to be 14, placing it at the lower limit of the high vulnerability category. Its conservation status has not been determined by the IUCN and this species is not listed by SEMARNAT. *Photo by Ferdinand Torres-Angeles.*



No. 40. *Kinosternon herrerae* (Stejneger, 1925). Herrera's Mud Turtle is distributed “in east-central Mexico, in southern Tamaulipas, eastern San Luis Potosí, northern Veracruz, Hidalgo, and Puebla” (Lemos-Espinal and Dixon 2013: 84). This individual was found at Laguna de Atezca, in the municipality of Molango de Escamilla. Wilson et al. (2013a) calculated its EVS as 14, placing it at the lower limit of the high vulnerability category. Its conservation status has been considered as Near Threatened by the IUCN, and is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

Plestiodon tetragrammus
Lepidophyma flavimaculatum
Boa imperator
Coluber constrictor
Drymobius chloroticus
Drymobius margaritiferus
Ficimia streckeri
Lampropeltis annulata
Leptophis mexicanus
Masticophis flagellum
Masticophis mentovarius
Mastigodryas melanolomus
Oxybelis aeneus
Pituophis catenifer
Pseudoelaphe flavirufa
Salvadora grahamiae
Spilotes pullatus
Tantilla rubra
Adelphicos quadrivirgatum
Amastridium sapperi
Coniophanes imperialis
Coniophanes piceivittis
Hypsiglena jani
Imantodes cenchoa
Imantodes gemmistratus
Leptodeira maculata
Ninia diademata
Pliocercus elapoides
Rhadinaea decorata
Sibon nebulatus
Tropidodipsas sartorii
Micrurus diastema
Rena dulcis
Storeria dekayi
Thamnophis marcianus
Scaphiodontophis annulatus
Bothrops asper
Crotalus scutulatus
Trachemys venusta
Kinosternon hirtipes
Kinosternon scorpioides

Clearly, a major conservation goal regarding the Hidalgo herpetofauna is to document the occurrence of these 122 species, which comprise 61.0% of the native herpetofauna, in one or more of the extant protected areas in the state, as well as determining what other areas could be designated in order to provide perpetual protection for the entire herpetofauna. Based on the distributions noted above, it is likely that such additional areas would need to be established in the Sierra Madre Oriental and the Mexican Plateau regions of the state.

Conclusions and Recommendations

Conclusions

A. Currently the herpetofauna of Hidalgo consists of 203 species, including 42 anurans, 17 salamanders, one

crocodilian, 137 squamates (44 lizards and 92 snakes), and six turtles.

B. The four physiographic regions recognized in Hidalgo harbor from 77 species in the Mexican Plateau to 166 in the Sierra Madre Oriental.

C. The number of species that are shared among physiographic regions varies from 13 between the Trans-Mexican Volcanic Belt and the Gulf Coastal Lowlands to 72 between the Sierra Madre Oriental and the Gulf Coastal Lowlands. The Coefficient of Biogeographic Resemblance values range from 0.14 between the Trans-Mexican Volcanic Belt and the Gulf Coastal Lowlands to 0.65 between the Mexican Plateau and the Trans-Mexican Volcanic Belt. The UPGMA dendrogram depicts two distinct clusters; one between the Mexican Plateau and Trans-Mexican Volcanic Belt (two montane regions), and another between the mountainous Sierra Madre Oriental and Gulf Coastal Lowlands. That the latter two regions cluster is predicated on them sharing a significant number of generalist species that typically occur on the Atlantic/Gulf versant from the southern USA, through Mexico and Central America, and into northern South America.

D. The level of herpetofaunal endemism in Hidalgo is relatively high. Of the 200 species making up the native herpetofauna, 108 (54.0%) are endemic to either the country of Mexico or the state of Hidalgo. Most of the endemic species are country endemics (104 or 96.3%), while only four are limited to the state of Hidalgo. All four of the state endemics are plethodontid salamanders of the genus *Chiropterotriton*.

E. The distribution status of the 203 species comprising the Hidalgo herpetofauna is as follows (in order of decreasing species numbers): country endemics (104; 51.2%); non-endemics (92; 45.3%); state endemics (four; 2.0%); and non-natives (three; 1.5%).

F. The principal environmental threats are deforestation, livestock, roads, pollution of water bodies, myths and other cultural factors, and diseases.

G. The conservation status of the Hidalgo herpetofauna was assessed using the SEMARNAT, IUCN, and EVS systems. As in prior MCS papers, the SEMARNAT system was found to be of minimal value, inasmuch as only 93 (46.5%) of the 200 native species have been evaluated by this system. Of the 93 species presently assessed, two are considered Endangered (P), 32 Threatened (A), and 59 Special Protection (Pr). A comparison of the SEMARNAT and distributional categorizations indicates that one of the two Endangered species is a non-endemic and the other is a country endemic species. Of the 32 Threatened species, nine are non-endemics and 23 are country endemics. Of the 59 Special Protection species,



No. 41. *Kinosternon integrum* LeConte, 1854. The Mexican Mud Turtle ranges from “central Sonora to the Río Verde in Oaxaca, but it also is widespread throughout the central and southern portion of the Mexican Plateau (Lemos-Espinal and Dixon 2013: 86–87). This individual was found in the Valle del Mezquital, in the municipality of Alfajayucan. Wilson et al. (2013a) determined its EVS as 11, placing it in the lower portion of the middle vulnerability category. Its conservation status has been ascertained as Least Concern by the IUCN, and it is placed in the Special Protection (Pr) category by SEMARNAT. *Photo by Christian Berriozabal-Islas.*

21 are non-endemics, 36 are country endemics, and two are state endemics.

H. The results of the IUCN assessment system (by category and proportion) are: CR (seven of 200 species; 3.5%); EN (14; 7.0%); VU (14; 7.0%); NT (seven; 3.5%); LC (122; 61.0%); DD (four; 2.0%); and NE (32; 16.0%).

I. In addition, application of the EVS system of conservation assessment to the 200 native species of Hidalgo, showed that the categorical values increase from low vulnerability (66 species; 33.0%) to medium (76; 38.0%), and then decrease to high vulnerability (58; 29.0%).

J. Comparing IUCN and EVS conservation status categorizations to one another showed that 60.3% of the EVS high vulnerability species are placed in one of the three IUCN threatened categories (CR, EN, or VU), and 54.1% of the low vulnerability species are in the LC category. As noted previously for other areas, the results of the application of these two conservation assessment systems to the Hidalgo herpetofauna do not correspond well with one another.

K. An examination of the conservation status of the species placed in the IUCN DD, NE, and LC categories demonstrates that many of these 158 species (79.0% of the 200 native species) have been evaluated inadequately compared to their respective EVS values, so we highly recommend these species be reevaluated to better indicate their prospects for survival.

L. The Relative Herpetofaunal Priority (RHP) measure was used to ascertain the conservation significance of

the four regional herpetofaunas in Hidalgo. This analysis indicates that the most significant regional herpetofauna is that of the Sierra Madre Oriental, as it contains the largest numbers of endemic species and high vulnerability species. The other three physiographic regions arranged in decreasing order of significance based on numbers of both endemic and high vulnerability species are: Trans-Mexican Volcanic Belt; Mexican Plateau; and Gulf Coastal Lowlands.

M. Only five protected areas are established in Hidalgo, all administered by the federal government. Collectively, the size of these areas comprises only 7.9% of the area of the state. Four of these five areas are located in the Trans-Mexican Volcanic Belt, which is only the second most significant physiographic region in the state. None of the areas is located in the Sierra Madre Oriental, which is by far the most significant region, based on the numbers of both endemic and high vulnerability species. Only two of the five areas feature the full array of necessary facilities. In addition, all five areas are occupied by landowners. Management plans, however, are available for four of the five areas. Herpetofaunal surveys are completed for only two of the five areas.

N. Collated herpetofaunal records for each of the five protected areas indicate that only 78 of the 203 species occupying the state have been recorded from the five areas combined. Of these 78 species, 51 are country endemics, which is 49.0% of the total of 104 such species in Hidalgo. Non-endemic species comprise 25 of 92 (27.2%) in the state. Only two of the four state endemic species (50.0%) are recorded and only in one of the protected areas. One good sign is that, to date, none of the three non-native species recorded in Hidalgo are known from any of the protected areas.

O. Future conservation efforts need to be directed toward establishing the presence of the remaining 122 herpetofaunal species in the existing system of protected areas, as well as determining what other protected areas could be developed in order to provide perpetual protection for the entire herpetofauna of Hidalgo. Presumably, such areas would need to be established in the Sierra Madre Oriental and the Mexican Plateau.

Recommendations

A. Our purpose for writing this eleventh entry in the Mexican Conservation Series is to document the composition, physiographic distribution, and conservation status of the 200 native species comprising the herpetofauna of Hidalgo. In examining the conservation status of these species, the EVS methodology placed them into low, medium, and high vulnerability categories in numbers which increased from 66 (low) to 76 (medium) and then decreased to

58 (high). The Relative Herpetofaunal Priority measure revealed that the most significant physiographic region in Hidalgo for conservation is the Sierra Madre Oriental, as it contains the most endemic species and species of high vulnerability. Unfortunately, there are no protected areas located within this region in Hidalgo, so the most fundamental conservation challenge is to correct this imbalance in the design of the protected area system in Hidalgo.

B. The next most important conservation challenge is to determine the presence in the existing protected areas of the 122 herpetofaunal species that have not been previously recorded in any of them and, beyond this step, to ascertain which additional protected areas could be established so that the entire native herpetofauna can be protected in perpetuity. Likely, such additional areas would need to be established in the Sierra Madre Oriental and Mexican Plateau regions.

C. After the presence of the entire native herpetofauna of Hidalgo in the protected areas system has been ascertained, then the next step will be to establish monitoring programs to guarantee the long-term survival of these creatures.

D. Such steps need to be taken with the greatest dispatch, as Hidalgo is the 17th most populous state in Mexico and the eighth most densely populated.

“At this point in the fight to solve the climate crisis, there are only three questions remaining: Must we change? Can we change? Will we change?”

—Al Gore (2017)

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Aurelio Ramírez-Bautista began his herpetological research career as an undergraduate student at the Los Tuxtlas Biological Field Station, Veracruz, Mexico. Aurelio received his Bachelor's in Biology from Universidad Veracruzana in Veracruz, Mexico, while his Master's in Science and doctorate were from the Universidad Nacional Autónoma de México (UNAM); and he received a postdoctoral appointment at the University of Oklahoma, Norman, Oklahoma, USA. His main research involves studies on ecology, demography, reproduction, conservation, and life history evolution, using amphibians and reptiles of Mexico as models. Aurelio was president of the Sociedad Herpetológica Mexicana and is currently Associate Editor of *Mesoamerican Herpetology*. Aurelio was a professor at UNAM, and is currently a professor at Universidad Autónoma del Estado de Hidalgo (UAEH) teaching population ecology, herpetology, and natural history of amphibians and reptiles. He has authored or co-authored 295 peer-reviewed papers and books on herpetology, ecology, life history evolution, sexual size dimorphism, reproduction, global climate change, potential distribution, demography, conservation, behavior, and thermal ecology. He has graduated 71 students (44 undergraduates, 18 Masters, and seven Ph.D. students); and served as an external advisor for Ph.D. students at Brigham Young University, University of Miami, and Eastern Carolina University, USA. Aurelio has received several national (Helia Bravo Hollis Award by the Technical Council of Scientific Research of UNAM, member of the National System of Researchers level II), and international awards (Donald Tinkle Award by Southwestern Association of Naturalists), and he has the profile PRODEP (Programa para el Desarrollo Profesional Docente) at UAEH.



Uriel Hernández-Salinas earned his Bachelor's, Master's and Ph.D. degrees at the Universidad Autónoma del Estado de Hidalgo. Uriel is a herpetologist and co-author of three books: *Herpetofauna del Valle de México: Diversidad y Conservación*, *Lista Anotada de los Anfibios y Reptiles del Estado de Hidalgo, México*, and *Los Anfibios y Reptiles del Estado de Hidalgo: Diversidad, Biogeografía y Conservación*. He is a full-time professor and curator-in-charge of the scientific collection of amphibians and reptiles at CIIDIR Durango. Uriel has authored or co-authored several peer-reviewed papers, and teaches Environmental Management II and Fauna Management in the master's and doctoral programs. In 2015, he became a member of the National System of Researchers, level 1. His main topics of interest are biodiversity, species richness, biogeography, and evolution of life histories of various species of the amphibians and reptiles of Mexico.



Raciél Cruz-Elizalde is a Mexican herpetologist who received his B.Sc. in Biology, and M.Sc. and Ph.D. in Biodiversity and Conservation, from the Universidad Autónoma del Estado de Hidalgo (UAEH). Raciél is interested in the ecology, life history evolution, diversity, and conservation of the amphibians and reptiles of Mexico. He has authored or co-authored several publications including papers, notes, book chapters and books about the ecology, life history evolution, sexual size dimorphism, reproduction, and conservation of amphibians and reptiles. Raciél's current research includes the life history evolution of diverse lizard species of genus *Sceloporus*, conservation issues in natural protected areas, and the analysis of ecological and morphological traits in the composition of amphibian and reptile assemblages.



Christian Berriozabal-Islas earned his bachelor's degree and his master's and Ph.D. degrees in the Biodiversity and Conservation program, all at Universidad Autónoma del Estado de Hidalgo. Christian is a herpetologist interested in species diversity, thermal ecology, functional diversity, climatic change, and distributional patterns using amphibians and reptiles as biological models. Currently, he is a professor at the Universidad Autónoma del Estado de Hidalgo. Christian has been involved in projects regarding environmental education and wildlife conservation in rural communities, was a co-author of the book *Los Anfibios y Reptiles del Estado de Hidalgo, México: Diversidad, Biogeografía y Conservación* (2014), and has authored or co-authored several papers on diversity, ecology, and climate change. He has a great interest in the natural history of the turtles of Mexico.



Israel Moreno-Lara is a herpetologist who recently graduated from the Universidad Autónoma del Estado de Hidalgo (UAEH) after completing a thesis project on the conservation, protection, and trafficking of arboreal lizards of the genus *Abronia* (Anguidae) under the mentorship of Aurelio Ramírez-Bautista and Raciél Cruz-Elizalde. Israel is working on the conservation of herpetofauna through educational projects using identification cards for the amphibian and reptile species of Hidalgo, and he is actively involved in scientific dissemination campaigns at UAEH.

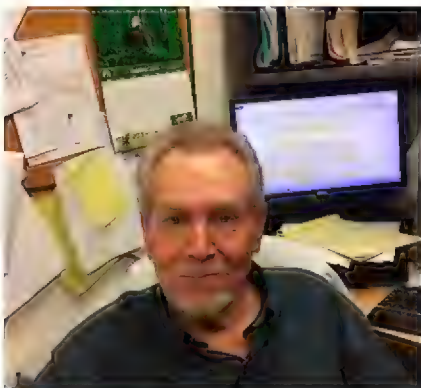


Dominic L. DeSantis is an Assistant Professor of Biology at Georgia College and State University, Milledgeville, Georgia, USA, in the Department of Biological and Environmental Sciences. Dominic's research interests broadly include the behavioral ecology, conservation biology, and natural history of herpetofauna. Much of his current research focusses on integrating multiple longitudinal monitoring technologies to study the proximate and ultimate drivers of spatial strategies and activity patterns in snakes. Dominic accompanied Vicente Mata-Silva, Elí García-Padilla, and Larry David Wilson on survey and collecting expeditions to Oaxaca in 2015, 2016, and 2017, and is a co-author on numerous natural history publications produced from those visits, along with an invited book chapter on the conservation outlook for herpetofauna in the Sierra Madre del Sur of Oaxaca. Overall, Dominic has authored or co-authored over 50 peer-reviewed scientific publications.

Herpetofauna of Hidalgo, Mexico



Eli García-Padilla is a herpetologist primarily focused on the ecology and natural history of the Mexican herpetofauna, particularly the Mexican states of Baja California, Tamaulipas, Chiapas, and Oaxaca. His first experience in the field was researching the ecology of the insular endemic populations of the rattlesnakes in the Gulf of California, and his Bachelor's degree thesis was on the ecology of *C. muertensis* (*C. pyrrhus*) on Isla El Muerto, Baja California, Mexico. To date, he has authored or co-authored over 100 peer-reviewed scientific publications. Eli is currently the formal Curator of Amphibians and Reptiles from Mexico in the electronic platform "Naturalista" of the Comisión Nacional para el Uso y Conocimiento de la Biodiversidad (CONABIO; <http://www.naturalista.mx>). One of his main passions is environmental education, and for several years he has been using audiovisual media to reach large audiences in promoting the importance of the knowledge, protection, and conservation of Mexican biodiversity. Eli's interests include wildlife and conservation photography, and his art has been published in several recognized scientific, artistic, and educational books, magazines, and websites. His present research project involves an evaluation of the jaguar (*Panthera onca*) as an umbrella species for the conservation of the herpetofauna of Nuclear Central America.



Jerry D. Johnson is Professor of Biological Sciences at The University of Texas at El Paso, USA, and he has extensive experience studying the herpetofauna of Mesoamerica, especially southern Mexico. Jerry is the Director of the 40,000-acre Indio Mountains Research Station, was a co-editor of *Conservation of Mesoamerican Amphibians and Reptiles* and co-author of four of its chapters. Jerry has authored or co-authored over 100 peer-reviewed papers and is the Mesoamerica/Caribbean editor for the Geographic Distribution section of *Herpetological Review*. One species, *Tantilla johnsoni*, has been named in his honor. Presently, he is an Associate Editor and Co-chair of the Taxonomic Board for the journal *Mesoamerican Herpetology*.



Vicente Mata-Silva is a herpetologist originally from Río Grande, Oaxaca, Mexico, whose interests include ecology, conservation, natural history, and biogeography of the herpetofaunas of Mexico, Central America, and the southwestern United States. Vicente received his B.S. degree from the Universidad Nacional Autónoma de México (UNAM), and his M.S. and Ph.D. degrees from the University of Texas at El Paso, USA (UTEP). Vicente is an Assistant Professor of Biological Sciences at UTEP in the Ecology and Evolutionary Biology Program, and Co-Director of UTEP's 40,000-acre Indio Mountains Research Station, located in the Chihuahuan Desert of Trans-Pecos, Texas. To date, Vicente has authored or co-authored over 100 peer-reviewed scientific publications. He also was the Distribution Notes Section Editor for the journal *Mesoamerican Herpetology*.



Larry David Wilson is a herpetologist with extensive experience in Mesoamerica. He was born in Taylorville, Illinois, USA, and received his university education at the University of Illinois at Champaign-Urbana (B.S. degree) and at Louisiana State University in Baton Rouge (M.S. and Ph.D. degrees). Larry has authored or co-authored more than 420 peer-reviewed papers and books on herpetology, including 18 papers from 2013–2019 on the EVS measure and the MCS surveys of the composition, distribution, and conservation status of the herpetofauna of different states in Mexico and other regions in Central America. Larry is the senior editor of *Conservation of Mesoamerican Amphibians and Reptiles* and a co-author of seven of its chapters. His other major books include *The Snakes of Honduras*, *Middle American Herpetology*, *The Amphibians of Honduras*, *Amphibians & Reptiles of the Bay Islands and Cayos Cochinos, Honduras*, *The Amphibians and Reptiles of the Honduran Mosquitia*, and *Guide to the Amphibians & Reptiles of Cusuco National Park, Honduras*. To date, he has authored or co-authored the descriptions of 71 currently recognized herpetofaunal species, and seven species have been named in his honor, including the anuran *Craugastor lauraster*, the lizard *Norops wilsoni*, and the snakes *Oxybelis wilsoni*, *Myriopholis wilsoni*, and *Cerrophidion wilsoni*. In 2005, he was designated a Distinguished Scholar in the Field of Herpetology at the Kendall Campus of Miami-Dade College. Currently, Larry is a Co-chair of the Taxonomic Board for the journal *Mesoamerican Herpetology*.



First record of *Raorchestes longchuanensis* Yang and Li, 1978 (Anura: Rhacophoridae) from northeastern Bangladesh suggests wide habitat tolerance

¹Hassan Al-Razi, ¹Marjan Maria, ¹Sabit Hasan, and ^{2,*}Sabir Bin Muzaffar

¹Faculty of Life and Earth Science, Department of Zoology, Jagannath University, Dhaka, BANGLADESH ²Department of Biology, United Arab Emirates University, Al Ain, UNITED ARAB EMIRATES

Abstract.—*Raorchestes* is a genus of small bush frog characterized by an absence of vomerine teeth, direct development without free swimming larvae, and a transparent gular pouch while calling. During a larger study on canopy fauna in the northeastern region of Bangladesh, five specimens of a small bush frog were collected from Satchari National Park in June and July 2017. This species was confirmed as *Raorchestes longchuanensis* using both morphometric and genetic analyses. Although this species was originally described from Yunnan, China, the authors speculated that it may be found in neighboring countries adjacent to the original records, including northern Myanmar, Thailand, Laos, and Vietnam. However, the current finding suggests that the species could be more widespread and resilient, spanning westwards through to northeastern India and Bangladesh. Data are also provided on coloration, habitat, natural history, and vocalizations of this little-known species. Although the species is designated Least Concern according to IUCN, more comprehensive studies should be undertaken to better understand its biology and population status to aid in a more comprehensive global conservation assessment.

Keywords. Amphibian, Asia, bush frog, DNA, range extension, Satchari National Park

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Introduction

Amphibians around the world are declining due to a variety of factors including habitat loss, competition with invasive species, emerging infectious diseases, and climate change (Blaustein et al. 2010; Fisher et al. 2009; Gray et al. 2009; Hayes et al. 2010; Pounds et al. 2006; Whittaker et al. 2013). Globally, nearly one-third of known amphibian species are threatened with extinction, and at least 42% of known amphibian species are experiencing population losses (Whittaker et al. 2013). These problems are acute in tropical Asia where large swaths of forest and wetland areas have already been replaced with agricultural land or urban environments, while existing patches are under constant pressure from human expansion. Thus, forest biodiversity within the tropical and subtropical belts has been the focus of major efforts in biodiversity conservation worldwide due to their recognized importance in climate stabilization, economic value (e.g., timber production or non-timber forest products), and to a lesser extent ecotourism potential (Muzaffar et al. 2011). Tropical forests in South

Asia have been classified variously into a range of tropical moist, tropical evergreen, and several other forest types based on the composition of vegetation (Champion and Seth 1968).

Recent work using phylogeographic approaches suggests that South Asian and Indo-Pacific forest types exhibit more compositional overlap than previously imagined (Slik et al. 2018). Incidentally, many of these areas are just beginning to be adequately explored for amphibian biodiversity and many species of forest-associated amphibians are being described (Biju and Bossuyt 2009; Biju et al. 2010a,b, 2011, 2014; Hasan et al. 2014a; Mahony 2011; Mahony et al. 2013, 2018; Purkayastha and Matsui 2012). Not surprisingly, many of these newly discovered species are already under some form of threat and could be heading towards extinction (IUCN 2015). Bangladesh hosts at least 64 species of anurans (Khan 2015), with several recently discovered new species (Hasan et al. 2012, 2014a; Howlader 2011; Howlader et al. 2015, 2016) and new country records (Hasan et al. 2010; Khan 2001; Mahony and Reza 2007a,b, 2008; Reza 2008), and several others are likely

Correspondence. * s_muzaffar@uaeu.ac.ae (ORCID: 0000-0001-9195-1677)

to be added to the list in the near future (Khan 2015).

Raorchestes Biju, Shouche, Dubois, Dutta, and Bossuyt (2010) is a genus of small-sized frogs with direct development in the Rhacophoridae family. A total of 62 *Raorchestes* species are known from India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Southern China, Vietnam, Cambodia, and West Malaysia (Frost 2019). Although four species are reported from Northeast India, only two species of this genus are found in Bangladesh (Frost 2019; IUCN 2015). Of the 62 *Raorchestes* species, 58 are from South Asia and only four species, including *Raorchestes longchuanensis* (Yang and Li 1979), occur in Southeast Asia and southern China (Frost 2019).

In this study, *Raorchestes longchuanensis* is reported from the north-eastern region of Bangladesh, based on observations made during a survey conducted as part of a larger study on canopy fauna in this region. Forests in the northeastern regions are considered to be tropical moist deciduous or tropical semi-evergreen forests (Champion and Seth 1968) and are located in the south and southwest of the Indian states of Meghalaya, Assam, and Nagaland, along with comparable forest types such as tropical moist deciduous and subtropical broad-leaved hill forests (Champion and Seth 1968). The objectives of this study were (i) to diagnose the presence of this frog species hitherto reported only from southern China; (ii) to provide morphometric, acoustic, and natural history data on this species; and (iii) to provide comments on its biogeography.

Methods

Study Area

Surveys were conducted in Satchari National Park (SNP), located in Habiganj District, Sylhet in northeastern Bangladesh. The park encompasses an area of about 243 ha (Mukul et al. 2010) and is located on Raghunandan hill, under Paikpara Union, Chunarughat Upazilla. The topography of this area is undulating with slopes and hillocks, ranging from 10–150 m in elevation. The climate is mainly tropical with high rainfall (300–800 mm) concentrated during the monsoon from June to September, with temperatures ranging from 25–32 °C. In the remaining dry period of the year, the region experiences occasional, low rainfall and lower temperatures of 12–25 °C (Mollah et al. 2004). The total annual average rainfall is 4,162 mm (Mollah et al. 2004). A number of small, sandy-bedded streams drain the forest during the rainy season. The maximum and minimum temperatures of the area are 32 °C and 12 °C, respectively, and the relative humidity fluctuates between 74% and 90% (Mollah et al. 2004).

Field Survey

Five specimens were collected in June and July 2017. Only acoustic searches were used to locate the calling

aggregations (Heyer et al. 1994). The temperature, humidity, habitat, perch height, and behavioral activities were recorded after encounter. All individuals collected were males since they were located based on their calls. No female individuals were found. The location of each collected specimen was recorded by a GPS device (Garmin eTrex 10), and temperature and humidity were recorded with a digital thermometer and hygrometer, respectively. Video footage of a calling male individual was also recorded using a DSLR camera (Nikon d7200 with 55–300 mm Vr lens). Five adult male individuals were collected from two different field surveys, fixed in formalin, and preserved in 70% ethanol. A small amount of thigh muscle was collected (as described below) from four specimens, and these tissue specimens were preserved in 70% ethanol. Specimens were deposited in the Museum of the Department of Zoology, Jagannath University, Dhaka, Bangladesh (reference numbers: JnUZool-A0117 to JnUZool-A0517).

Morphometrics

The following morphometric measurements of the specimens were taken before preservation with a digital caliper (to the nearest 0.1 mm): snout-vent length, from tip of snout to vent (SVL); head length, distance between tip of snout and rear of mandible (HL); head width, at angle of jaw (HW); eye diameter, horizontal diameter of eye (ED); tympanum diameter, maximum diameter of tympanum (TD); eye-nostril distance, distance between anterior canthus of eye and nostril (END); snout length, from anterior canthus of eye to tip of snout (SL); inter orbital distance, least distance between proximal edges of upper eyelids (IOD); internarial distance, least distance between nostrils (IND); upper eyelid width, maximum transverse distance of upper eyelid measured from inner edge to outer edge (UEW); eye-tympanum distance, least distance between eye and tympanum (ETD); thigh length, distance from middle of vent to knee (THL); shank length, distance between knee and heel (SL); foot length, from base of inner metatarsal tubercle to tip of Toe IV (FL); hand length, from base of outer palmar tubercle to tip of Finger IV (HAL); width of 1st to 4th finger disks, measured at the widest point on the finger disk (FinDW); lengths of 1st to 4th fingers, from base of palm to tip of respective finger (FinL, F-I to F-IV); lengths of 1st to 5th toes, from inner metatarsal tubercle region to tip of respective toe (ToeL, T-I to T-V); and width of 1st to 5th toe disks, greatest horizontal distance between edges of Toe I disks (ToeDW).

DNA Extraction and Amplification

The procedure described in Vences et al. (2012) was followed for DNA extraction. Approximately 1.5 mm² of thigh muscle tissue was excised from each specimen for extraction. The PCR amplification and sequencing of the



Fig 1. External features of *Raorchestes longchuanensis*. (A) Calling position of an adult male individual where transparent gular sack is prominent; (B) Ventral aspect of hand with rounded, disked fingertips; (C) Ventral aspect of foot with rounded disked, reddish toe tips.

16S rRNA gene were performed following Palumbi et al. (1991) and Bossuyt et al. (2004), respectively. Primers of 5'-GCCTGTTTATCAAAAACAT-3' (16Sar-L) and 5'-CCGGTCTGAACTCAGATCACGT-3' (16Sbr-H) as forward and reverse primers for 16S (Palumbi et al. 1991), were used for this study. PCR amplifications were performed in a 20 µl reaction volume with the following cycling conditions: an initial denaturing step at 95 °C for 3 min; 40 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s, and extending at 72 °C for 45 s; and a final extending step of 72 °C for 5 min. The amplified products were sent to 1st Base Laboratories, Malaysia, for sequencing. The sequences were checked manually using the program Chromas lite 2.01 (http://www.technelysium.com.au/chromas_lite.html). The sequences have been submitted to GenBank (Accession nos: MH699074, MN193412, MN193413, and MN193414).

To infer the phylogenetic position of the current species, homologous sequences for 16S rRNA genes of 24 species were downloaded from the NCBI GenBank database (Table 1). Sequences were aligned using the MUSCLE tool in MEGA X (Kumar et al. 2018), and alignments were checked visually and corrected manually when necessary. Alignment gaps were treated as missing data. The best fit model for nucleotide substitution was selected from 24 models available in MEGA X (Kumar et al. 2018) based on the minimum Akaike Information Criterion (AIC) value (Posada and Crandall 2001). Maximum Likelihood was performed in MEGA X. The general time reversal nucleotide substitution model with

gamma distribution with Invariant Sites (GTR+G+I), obtained as a best fit model in the model test (AIC = 3932.32, lnL = -1903.84), was used for constructing the phylogenetic tree based on the maximum likelihood method by using 1,000 bootstrap replicates in MEGA X (Kumar et al. 2018). Interspecific and intraspecific mean uncorrected pairwise distances were computed in MEGA X (Kumar et al. 2018).

Call Recording and Analysis

The call of a single male individual (JnUZool- A0317) was recorded with a DSLR camera (Nikon d7200 with 55–300 mm VR lens) on 2 August 2017. The sound was extracted from the video clip by using Windows Movie Maker 2012 software. The microphone was approximately 1–1.5 m away from the calling male. Air temperature and humidity were taken with a digital hygrometer. Call analysis used Raven Pro Ver. 1.5 (Charif et al. 2010; Bioacoustics Research Program 2011). Measured parameters were call-group duration, inter-call group interval, duration of intervals between pulses, call duration, and call rate of a call bout comprised of nine call groups.

Results

First Observation

During the surveys, this species was observed for the first time on 5 June 2017. The day was cloudy and after a rainfall before dusk (1810 h to 1840 h) the surveyors heard several *tik-tik-tik-tik* calls from the bamboo bushes, not very far from the narrow road of the SNP visitor's watch tower. The bamboo bushes were entered and the search for the frogs commenced. As there was still day light, the search for the frog was conducted without any light. After more than 20 min an adult male individual was found on a bamboo tree. The male was calling continuously while keeping its anterior part angled downwards (Fig. 1A). The position of the frog was 1.5 m above the ground. The individual was observed for a few minutes, then collected as a sample specimen.

Species Identification

The species was initially identified as *Raorchestes longchuanensis* based on the limited morphological characters described by Yang and Li (1978) in their original description of the species. *Raorchestes longchuanensis* is a small species in which adult males are 22.9 ± 0.9 mm ($n = 5$), one-fourth of the toes are webbed, and the disk of the third finger is smaller than the tympanum diameter. This specimen was confirmed as *R. longchuanensis* using the BLAST technique with a sequence similarity of more than 99% (492 of 496 bp). Identification was also confirmed with Maximum

Table 1. 16S rRNA sequences for *Raorchestes* species and *Kurixalus eiffingeri* from GenBank, with accession numbers and localities. SNP = Satchari National Park.

	Species	GenBank accession no.	Locality	Source
01	<i>R. longchuanensis</i>	MH699074	SNP, Bangladesh	This study
02	<i>R. longchuanensis</i>	MN193412	SNP, Bangladesh	This study
03	<i>R. longchuanensis</i>	MN193413	SNP, Bangladesh	This study
04	<i>R. longchuanensis</i>	MN193414	SNP, Bangladesh	This study
05	<i>R. longchuanensis</i>	GQ285675	China	Yu et al. 2010
06	<i>R. ghatei</i>	KF366385	Western Ghats, India	Padhye et al. 2013
07	<i>R. gryllus</i>	AB933309	Vietnam	Nguyen et al. 2014
08	<i>R. shillongensis</i>	MG980282	Meghalaya, India	Unpublished
09	<i>R. shillongensis</i>	MG980283	Meghalaya, India	Unpublished
10	<i>R. parvulus</i>	MH590204	Gunung Jerai, Malaysia	Chan et al. 2018
11	<i>R. menglaensis</i>	EU924621	Yunnan, China	Yu et al. 2009
12	<i>R. tuberothumerus</i>	KP137388	Western Ghats, India	Padhye et al. 2015
13	<i>R. bombayensis</i>	KF767502	Castlerock village, Karnataka, India	Padhye et al. 2013
14	<i>R. bombayensis</i>	EU450019	Castlerock village, Karnataka, India	Biju and Bossuyt 2009
15	<i>R. indigo</i>	KM596557	Western Ghats, India	Vijayakumar et al. 2014
16	<i>R. ponmudi</i>	EU450026	Ponmudi, Western Ghats, India	Biju and Bossuyt 2009
17	<i>R. ponmudi</i>	KM596576	Ponmudi, Western Ghats, India	Vijayakumar et al. 2014
18	<i>R. aureus</i>	KM596540	Western Ghats, India	Vijayakumar et al. 2014
19	<i>R. montanus</i>	KM596552	Western Ghats, India	Vijayakumar et al. 2014
20	<i>R. signatus</i>	KM596561	Western Ghats, India	Vijayakumar et al. 2014
21	<i>R. marki</i>	JX092719	Western Ghats, India	Vijayakumar et al. 2014
22	<i>R. charius</i>	KU169985	Karnataka, India	Biju et al. 2016
23	<i>R. beddomii</i>	EU449998	Western Ghats, India	Biju and Bossuyt 2009
24	<i>R. chalazodes</i>	KJ619641	Western Ghats, India	Das 2015
25	<i>R. dubois</i>	JX092668	Kodaikanal, India	Vijayakumar et al. 2014
26	<i>R. munnarensis</i>	JX092655	Western Ghats, India	Vijayakumar et al. 2014
27	<i>R. chromasynchysi</i>	KM596543	Western Ghats, India	Vijayakumar et al. 2014
28	<i>Kurixalus eiffingeri</i>	DQ468673	Okinawa Islands, Japan	Wu et al. 2016

likelihood phylogenetic analyses (Fig. 2) and the intra- and interspecific genetic *p*-distances (0.2–0.3% and 5.0–13.9% average values, respectively, Table 3).

Morphometrics

Snout-vent lengths of the five adult males ranged from 21.4–23.9 mm. Head width was larger than the length of head (HW = 7.8–9.1 mm, HL = 6.30–7.17 mm). The snout was pointed in the dorsal view; snout length was larger than the eye diameter (SL = 2.90–3.12 mm, ED = 2.50–2.86 mm); nostrils were oval and closer to snout tip than to eye; vomerine teeth were absent; eyes were small with a horizontal pupil. Fingers were web-less and finger tips had disks that were rounded. Relative lengths of fingers were as follows: I < II < IV < III (Table 2). The thigh was slightly longer than the tibia (TL = 11.20–12.10 mm, THL = 11.70–12.50 mm); Toe V was longer than Toe III; relative lengths of toes were as follows: I < II < III < V < IV (Table 2). Subarticular tubercles were prominent and rounded; outer metatarsal tubercles were absent while inner metatarsal tubercles were present; and toe tips possessed distinct, rounded disks (Fig. 1B–C).

Coloration

Considerable color variation was recorded in this species, including four different body colors in the specimens that could change in relation to the environment. A distinct or faint “)(” mark was always present on the dorsal surface of the body (Fig. 3). Sometimes a narrow yellow mid-dorsal line was present on the dorsal side (in one of five specimens). Vocal sac was external, single, and transparent with white spotting. The discs of the fingers and toes were reddish, orange, or whitish. There was a pale or prominent brown or black line between the upper eyelids. The dorsum was of a dark chocolate, dark brown, or pale brown coloration in the live specimens. The dorsal coloration and the pale flanks became gray or light brown to dark brown after preservation. Eyelids and tympanic fold were blackish or brown (Fig. 3).

Habitat, Natural History, and Vocalization

The specimens of *Raorchestes longchuanensis* were found in bushes inside the forest, and sometimes near road sides or near human habitations. Usually they were

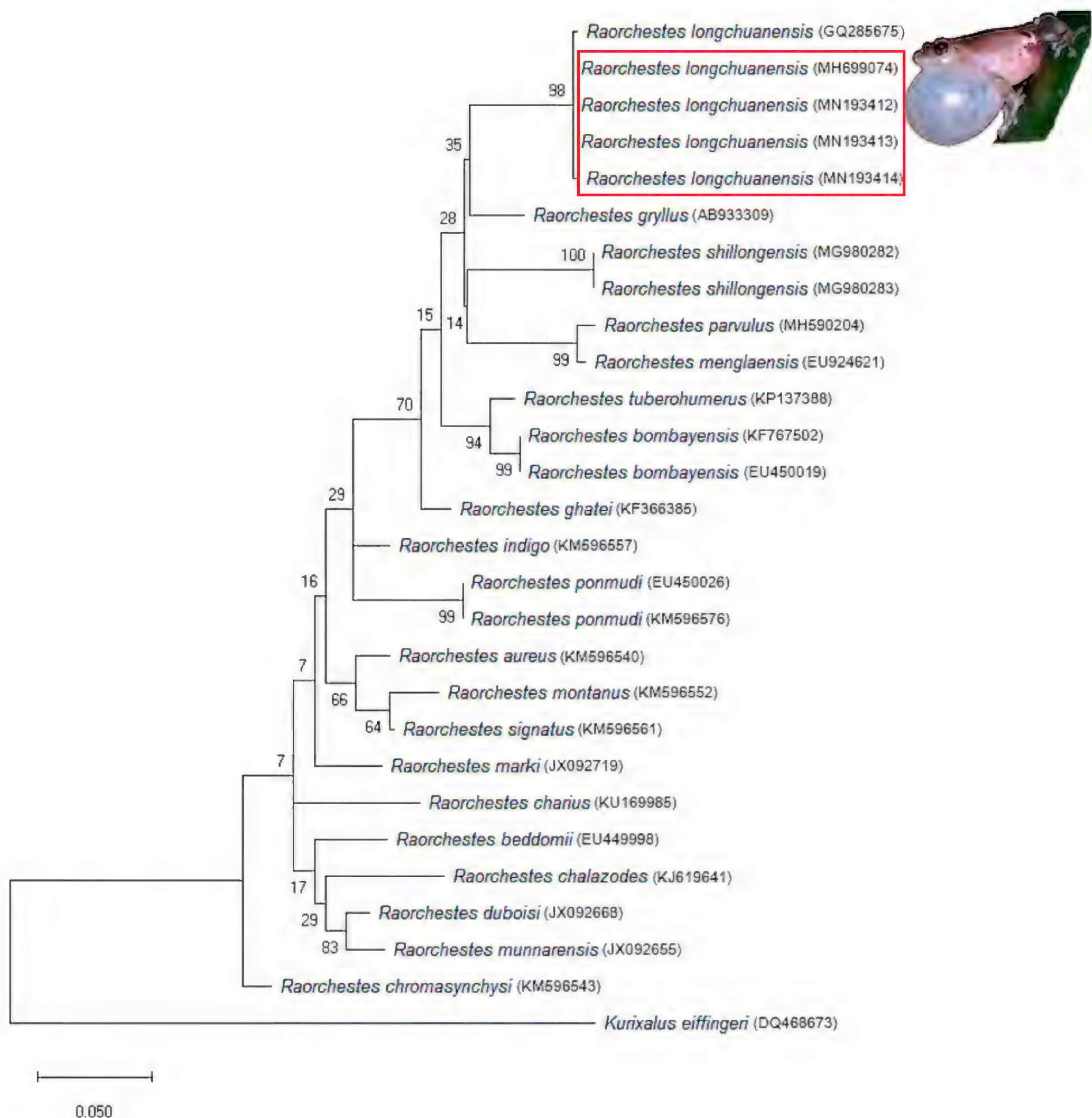


Fig 2. Maximum likelihood phylogenetic tree based on 16S rRNA genes, showing the identity of the specimens as *Raorchestes longchuanensis*. The red box shows the specimens from the present study.

found living amongst the bamboo bushes and Calamus palm bushes (*Calamus* spp.). Individuals usually were perched on leaves and branches of small trees or bamboo bushes. All individuals were found less than 2 m above the ground. *Raorchestes longchuanensis* became active with the onset of rain in the month of April. Breeding males started calling from dusk and continued until 2400 h. During rain and in cloudy environments they became active in the day time.

Advertisement calls occurred in call groups (Fig. 4). The duration of the analyzed call was 20 s. The number of call groups within this call was nine, and the number of pulses varied from three to six. The duration of each call group was 0.526 ± 0.142 s ($n = 9$) and it varied with

the number of calls. The interval between call groups was 1.831 ± 0.403 s ($n = 8$). Pulse duration was 0.013–0.024 s (0.018 ± 0.003 s, $n = 42$ pulses), and the duration of intervals between pulses was 0.102–0.163 s (0.124 ± 0.013 s, $n = 33$ intervals). The lowest frequency was 1.35 kHz and the highest frequency was 4.07 kHz. The overall peak frequency of the calls was 2.76 kHz. The air temperature was 27.4 °C at the time of the call.

Discussion

Several species of frogs have been recorded for the first time or described from Bangladesh in recent years (Asmat 2007; Hasan et al. 2012, 2014a,b; Howlader

Table 2. Morphologic measurements (mm) of *Raorchestes longchuanensis* specimens collected from Satchari National Park, Bangladesh ($n =$ five males).

Characters	Abbreviation	JnUZool-A0117	JnUZool- A0217	JnUZool- A0317	JnUZool- A0117	JnUZool-A0418
Snout-vent length	SVL	22.60	23.90	21.40	23.89	22.52
Head length	HL	6.80	7.17	6.40	7.03	6.80
Head width	HW	8.80	9.10	8.69	9.00	8.00
Eye diameter	ED	2.75	2.86	2.50	2.71	2.66
Tympanum diameter	TD	1.50	1.56	1.43	1.6	1.49
Eye-nostril distance	EN	2.15	2.22	1.88	2.20	1.96
Snout length	SL	3.12	3.10	2.90	3.07	3.03
Interorbital distance	IOD	3.18	3.52	3.28	3.40	3.40
Internarial distance	IND	2.35	2.50	2.25	2.47	2.34
Upper eyelid width	UEW	1.32	1.61	1.25	1.60	1.28
Eye-tympanum distance	ETD	0.72	0.82	0.70	0.75	0.71
Thigh length	THL	12.34	12.50	11.70	12.41	12.26
Tibia length	TL	12.00	12.10	11.20	12.08	12.00
Foot length	FL	8.90	9.56	8.83	9.53	9.03
Hand length	HAL	5.72	6.21	5.69	6.17	6.06
Forearm length	FLL	5.8	6.42	5.61	6.33	6.00
Finger I disk width	Fin1DW	0.50	0.56	0.47	0.56	0.48
Finger II disk width	Fin2DW	0.80	0.85	0.79	0.87	0.80
Finger III disk width	Fin3DW	1.30	1.47	1.26	1.56	1.31
Finger IV disk width	Fin4DW	1.23	1.25	1.18	1.25	1.22
Finger I length	Fin1L	1.61	1.93	1.6	1.92	1.59
Finger II length	Fin2L	2.20	2.38	1.92	2.33	2.17
Finger III length	Fin3L	4.14	4.36	4.04	4.31	4.15
Finger IV length	Fin4L	2.50	2.52	2.35	2.53	2.47
Toe I length	Toe1L	3.24	3.25	2.87	3.32	3.20
Toe II length	Toe2L	4.51	4.56	4.52	4.62	4.50
Toe III length	Toe3L	6.96	6.99	6.93	7.08	6.95
Toe IV length	Toe4L	8.71	8.98	8.69	9.04	8.68
Toe V length	Toe5L	6.35	6.08	6.77	6.96	6.30
Toe I disk width	Toe1DW	0.52	0.58	0.43	0.53	0.50
Toe II disk width	Toe2DW	0.80	0.77	0.74	0.82	0.72
Toe III disk width	Toe3DW	0.88	0.87	0.82	0.90	0.85
Toe IV disk width	Toe4DW	1.18	1.26	1.11	1.30	1.21
Toe V disk width	Toe5DW	0.86	0.98	0.76	1.02	0.87



Fig 3. Color variation in *Raorchestes longchuanensis*. (A) Pale brown dorsum with faint “(”) mark; (B) Dark brown dorsum with prominent “(”) mark; (C) Dark chocolate color dorsum without mid-dorsal line; (D) Dark chocolate color dorsal side with narrow yellow mid-dorsal line.

2011; Howlader et al. 2015, 2016; Khan 2015; Mahony and Reza 2007a,b, 2008; Reza 2008; Mahony et al. 2009). This is not surprising since Bangladesh is a small country situated on the boundary of two major ecological realms, with the Indomalayan realm encompassing most of Bangladesh and the beginning of the Palearctic realm to the north (Udvardy 1975). The position of Bangladesh on the edge of these biogeographic zones makes it prone to hosting biota from both realms. These areas have been also recognized as part of the Indo-Burma global biodiversity hotspot, with significant abundance of threatened and regionally endemic amphibians (Sechrest et al. 2002; Jenkins et al. 2013). Until recently, only 49 species of amphibians were officially recorded from Bangladesh (Hasan et al. 2014b). The recent rise in new amphibian records constitutes an almost 65% increase

in the anurans of Bangladesh since the late 1990s, suggesting that exploration of the region’s amphibians is just beginning (calculated from IUCN 2015; Khan 2015).

The tree frogs of Bangladesh have been particularly under-studied. One species in the genus *Philautus* was originally recorded from Bangladesh (Hasan and Feeroz 2014). This genus was originally considered to be distinguishable from others by the absence of vomerine teeth and its occurrence in South and Southeast Asia (Bossuyt and Dubois 2001). However, further study showed that *Philautus* exhibits diverse morphological traits and a wide variety of life history strategies (Bossuyt and Dubois 2001). *Philautus* from the Western Ghats could be separated from *Pseudophyllautus* (Sri Lankan sister clade) and appeared to be distinct from those in northeastern India. This led to the erection of a

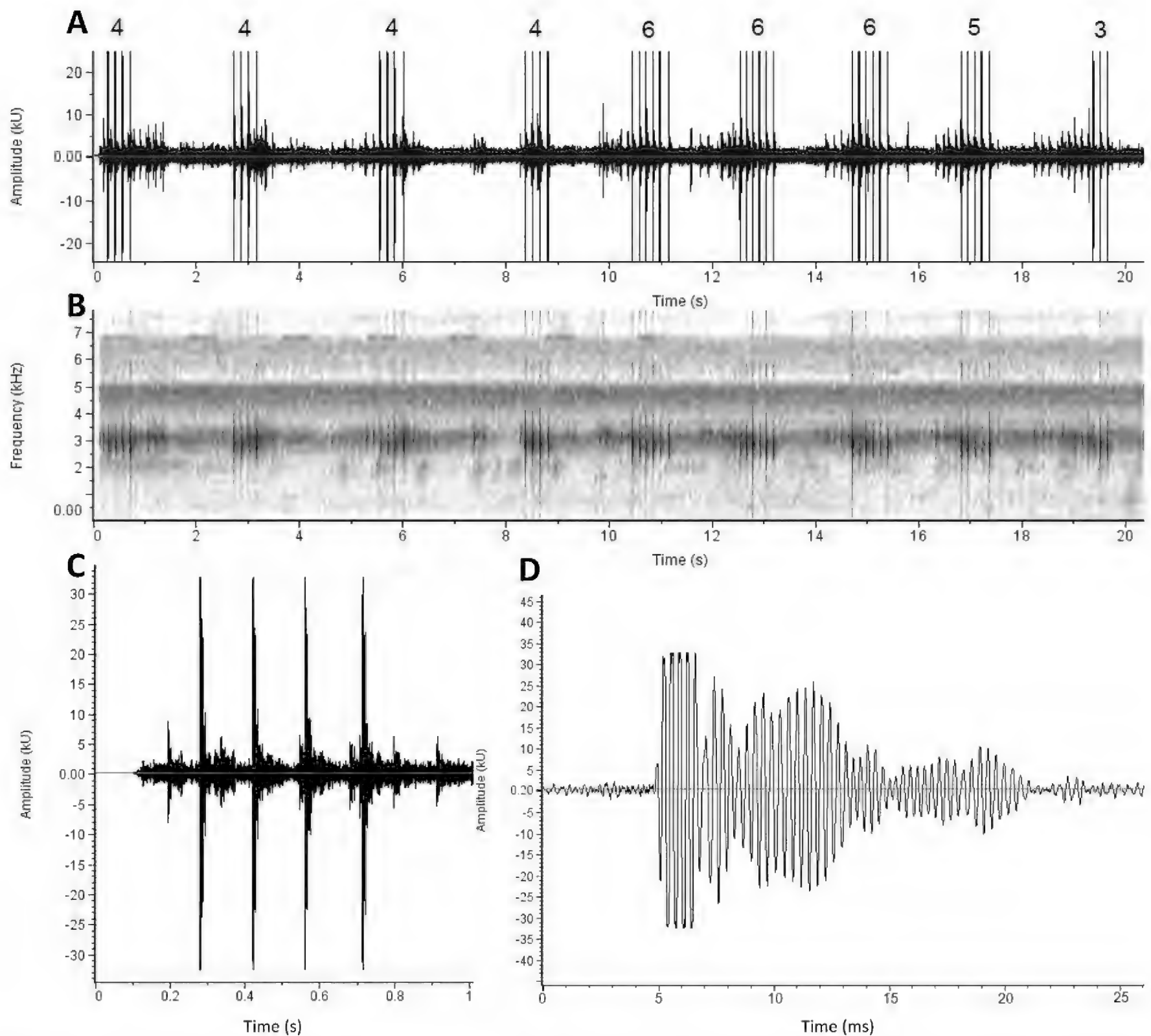


Fig 4. Advertisement call of *Raorchestes longchuanensis* at ambient air temperature 27.4 °C. (A) A call consists of five call groups, numbers on the top indicate pulse numbers in the respective call group. (B) Spectrogram of the call. (C) First call group with four pulses. (D) A pulse of the 5th call group.

new genus *Raorchestes* (based on molecular phylogeny), consisting of small frogs with no vomerine teeth, direct development without free swimming larvae, and a transparent gular pouch while calling (Biju et al. 2010).

At least 62 *Raorchestes* species have been named to date, but many of these are species complexes and further study could reveal cryptic species (Frost 2019). Many of the *Philautus* species from across the distribution of this genus have become subsumed into *Raorchestes* (Ghose and Bhuiyan 2012; IUCN 2015). This includes the former *P. annandalii* (Darjeeling Bush Frog) being changed to *R. annandalii*, also recorded from the northeastern regions of Bangladesh (IUCN 2015; Khan 2015, 2018). The second species, *R. parvulus* (Karin Bubble-nest Frog) reported by Mahony et al. (2009) and later by Ghose and Bhuiyan (2012), is considered to be a possible member of a species group found in southeastern Bangladesh (Mahony and Reza 2007a,b; Mahony et al. 2009; Khan 2015; IUCN 2016).

Raorchestes longchuanensis was previously

considered endemic to southern China, restricted to some prefectures in Yunnan province at 1,150–1,600 m above sea level, adjacent to northern and northeastern Myanmar (Yang et al. 2004). However, this species has already been recorded from Vietnam (Orlov et al. 2002). Yang et al. (2004) have suggested that the species was likely to occur in neighboring countries including northern Myanmar, Thailand, and Laos. The species has escaped detection in some areas possibly due to its cryptic nature and the general absence of major exploration in these regions.

The findings reported here also point to the broader floral similarities between Myanmar and northeastern India (including states such as Tripura, Mizoram, Manipur, and Nagaland) [Slik et al. 2018], which forms a belt connecting our study site, and northern Myanmar across into Yunnan, China. This study presents a range extension of this species of approximately more than 600 km in the western direction from its type locality (Fig. 5). An earlier study showed a range extension of

Table 3. Intraspecific and interspecific genetic divergence. Uncorrected *p*-distance between 16S rDNA sequences of 20 closely related species of *Raorchestes* and *Kurixalus eiffingeri*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 <i>R. longchuanensis</i> (GQ285675)	--																											
2 <i>R. longchuanensis</i> (MH699074)	0.002	--																										
3 <i>R. longchuanensis</i> (MN193412)	0.005	0.002	--																									
4 <i>R. longchuanensis</i> (MN193413)	0.002	0.000	0.002	--																								
5 <i>R. longchuanensis</i> (MN193414)	0.002	0.000	0.002	0.000	--																							
6 <i>R. ghatei</i> (KF366385)	0.050	0.048	0.050	0.048	0.048	--																						
7 <i>R. gryllus</i> (AB933309)	0.050	0.048	0.050	0.048	0.048	0.045	--																					
8 <i>R. shillongensis</i> (MG980282)	0.075	0.073	0.075	0.073	0.073	0.061	0.055	--																				
9 <i>R. shillongensis</i> (MG980283)	0.075	0.073	0.075	0.073	0.073	0.061	0.055	0.000	--																			
10 <i>R. bombayensis</i> (KF767502)	0.059	0.057	0.059	0.057	0.057	0.043	0.045	0.070	0.070	--																		
11 <i>R. bombayensis</i> (EU450019)	0.059	0.057	0.059	0.057	0.057	0.043	0.045	0.070	0.070	0.000	--																	
12 <i>R. parvulus</i> (MH590204)	0.066	0.064	0.066	0.064	0.064	0.048	0.059	0.070	0.070	0.061	0.061	--																
13 <i>R. tuberohumerus</i> (KP137388)	0.061	0.059	0.061	0.059	0.059	0.041	0.048	0.068	0.068	0.020	0.020	0.064	--															
14 <i>R. beddomii</i> (EU449998)	0.077	0.075	0.077	0.075	0.075	0.066	0.080	0.091	0.091	0.080	0.080	0.073	--															
15 <i>R. chalazodes</i> (KJ619641)	0.105	0.102	0.105	0.102	0.102	0.089	0.091	0.098	0.098	0.082	0.082	0.077	0.084	0.059	--													
16 <i>R. aureus</i> (KM596540)	0.075	0.073	0.075	0.073	0.073	0.059	0.075	0.082	0.082	0.064	0.064	0.080	0.066	0.057	0.066	--												
17 <i>R. charius</i> (KU169985)	0.093	0.091	0.091	0.091	0.091	0.070	0.089	0.093	0.093	0.080	0.080	0.066	0.077	0.055	0.068	0.066	--											
18 <i>R. chromasynchysi</i> (KM596543)	0.086	0.084	0.086	0.084	0.084	0.061	0.084	0.080	0.080	0.075	0.075	0.068	0.086	0.052	0.055	0.050	0.057	--										
19 <i>R. duboisi</i> (JX092668)	0.086	0.084	0.086	0.084	0.084	0.066	0.068	0.080	0.080	0.068	0.068	0.073	0.075	0.043	0.048	0.052	0.061	0.043	--									
20 <i>R. indigo</i> (KM596557)	0.061	0.059	0.061	0.059	0.059	0.041	0.061	0.075	0.075	0.059	0.059	0.066	0.061	0.064	0.073	0.036	0.066	0.055	0.052	--								
21 <i>R. marki</i> (JX092719)	0.070	0.068	0.070	0.068	0.068	0.066	0.073	0.080	0.080	0.066	0.066	0.073	0.059	0.061	0.057	0.045	0.057	0.050	0.048	0.043	--							
22 <i>R. menglaensis</i> (EU924621)	0.059	0.057	0.059	0.057	0.057	0.050	0.057	0.068	0.068	0.059	0.059	0.011	0.061	0.082	0.084	0.082	0.068	0.070	0.075	0.064	0.070	--						
23 <i>R. montanus</i> (KM596552)	0.086	0.084	0.086	0.084	0.084	0.066	0.086	0.093	0.093	0.070	0.070	0.082	0.077	0.059	0.061	0.034	0.064	0.043	0.050	0.048	0.055	0.084	--					
24 <i>R. munnarensis</i> (DC092655)	0.091	0.089	0.091	0.089	0.089	0.070	0.082	0.086	0.086	0.080	0.080	0.082	0.073	0.041	0.057	0.057	0.068	0.048	0.023	0.061	0.048	0.084	0.055	--				
25 <i>R. ponmeidi</i> (KM596576)	0.068	0.066	0.068	0.066	0.066	0.057	0.066	0.093	0.093	0.073	0.073	0.075	0.075	0.055	0.089	0.057	0.070	0.068	0.064	0.050	0.066	0.077	0.064	0.066	--			
26 <i>R. ponnudi</i> (EU4511026)	0.068	0.066	0.068	0.066	0.066	0.057	0.066	0.093	0.093	0.073	0.073	0.075	0.075	0.055	0.089	0.057	0.070	0.068	0.064	0.050	0.066	0.077	0.064	0.066	0.000	--		
27 <i>R. signatus</i> (KM596561)	0.086	0.084	0.086	0.084	0.084	0.066	0.084	0.084	0.084	0.075	0.075	0.077	0.077	0.048	0.050	0.025	0.061	0.041	0.043	0.041	0.048	0.080	0.020	0.048	0.061	0.061	--	
28 <i>Kurixalus eiffingeri</i> (DQ468673)	0.139	0.141	0.139	0.141	0.141	0.141	0.141	0.148	0.148	0.132	0.132	0.157	0.134	0.130	0.139	0.148	0.141	0.132	0.136	0.148	0.134	0.155	0.148	0.132	0.145	0.145	0.141	--

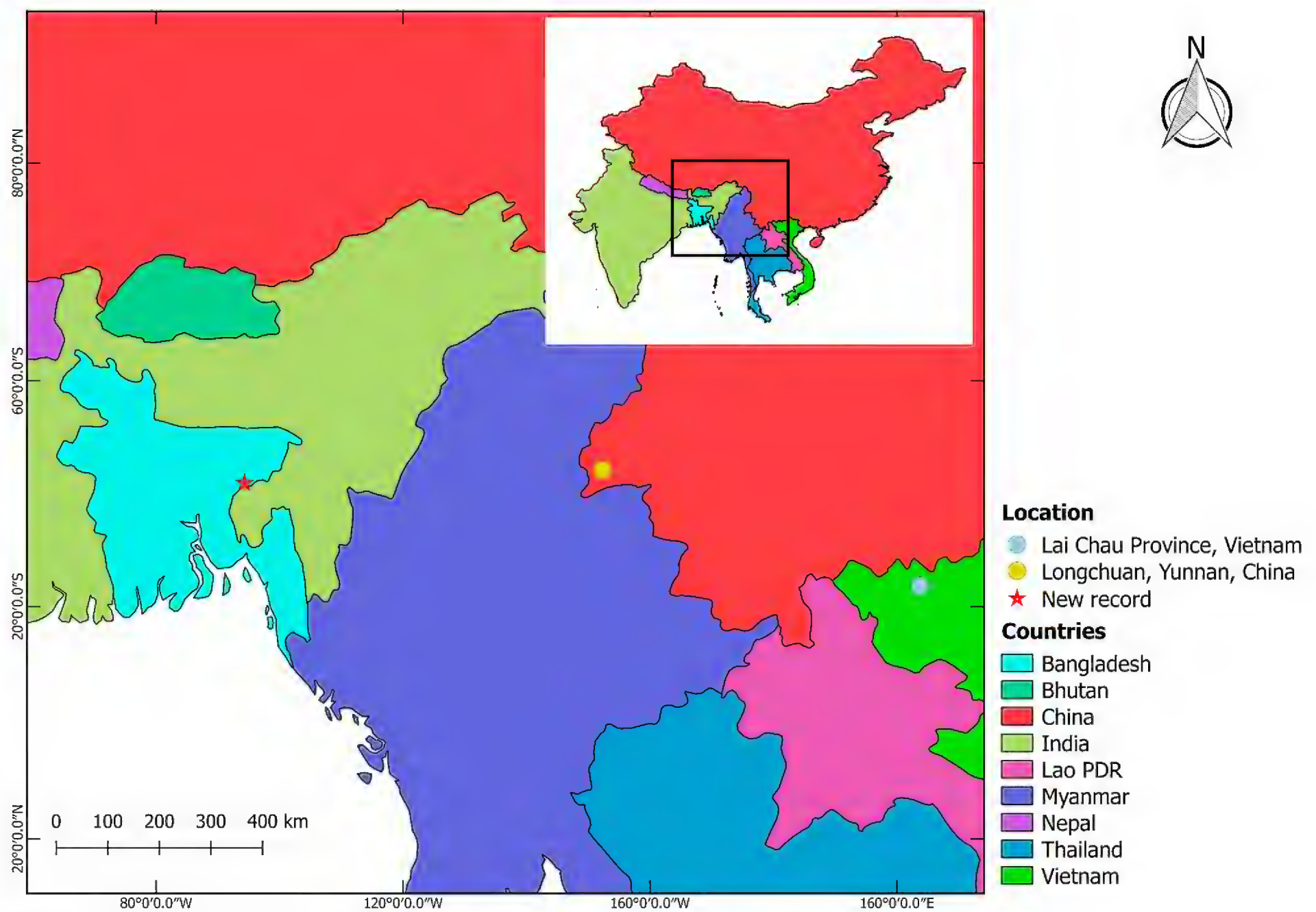


Fig 5. Map showing the previously reported and present records of *Raorchestes longchuanensis*.

more than 600 km in the east for this species (Orlov et al. 2002). Thus, we suspect that the species has a much wider distribution, and further studies in these regions will confirm its occurrence. We hope that more studies, especially those targeting arboreal species, will continue in Bangladesh and in neighboring regions to document and potentially save these species from the risks posed by the perpetual exploitation of timber in these regions. A comprehensive effort needs to be made to at least safeguard the remaining wilderness areas in Bangladesh and in neighboring regions.

Conclusions

This study adds *Raorchestes longchuanensis* as a new species to the anuran fauna of Bangladesh. Although currently assessed as Least Concern by the IUCN (Yang et al. 2004), its geographic distribution and current conservation status need to be re-evaluated, since its habitats have further degraded since the last assessment. The breeding biology, ecology, and habitat characteristics of the species need to be determined to ensure long-term conservation of this species in Bangladesh and elsewhere.

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Hassan Al-Razi has a B.Sc. in Zoology and an M.Sc. in Wildlife and Biodiversity Conservation from the Department of Zoology, Jagannath University, Dhaka, Bangladesh. Currently, Hassan is a researcher at the same university, and he has been working on nocturnal animals for more than four years. His research fields include the diversity, taxonomy, and breeding biology of herpetofauna, as well as primate and small carnivore conservation.



Marjan Maria is a B.Sc. student in the Department of Zoology, Jagannath University, Dhaka, Bangladesh. Marjan is a wildlife researcher interested in the diversity, taxonomy, and ecology of herpetofauna, as well as nocturnal mammals and birds.



Sabit Hasan is an M.Sc. student in the Department of Zoology, Jagannath University, Dhaka, Bangladesh. Sabit is currently involved in the study of various nocturnal animals. He is interested in the ecology and conservation of wildlife.



Sabir Bin Muzaffar is a Professor in the Department of Biology, United Arab Emirates University. Sabir is an ecologist with broad interests in animal movement, migration, community structure, and conservation.



Geographic range extension for the Lobatse Hinge-back Tortoise, *Kinixys lobatsiana* (Power, 1927), with first records from the Soutpansberg region

^{1,*}Flora Ihlow, ²Ryan Van Huyssteen, ¹Melita Vamberger, ³Dawn Cory-Toussaint, ⁴Margaretha D. Hofmeyr, and ¹Uwe Fritz

¹Museum of Zoology, Senckenberg Dresden, A.B. Meyer Building, 01109 Dresden, GERMANY ²Soutpansberg Centre for Biodiversity and Conservation, Medike Nature Reserve, Soutpansberg, SOUTH AFRICA ³University of Venda Limpopo, SOUTH AFRICA ⁴Chelonian Biodiversity and Conservation, Department of Biodiversity and Conservation Biology, University of the Western Cape, Bellville 7535, SOUTH AFRICA (deceased)

Abstract.—The Lobatse Hinge-back Tortoise, *Kinixys lobatsiana* (Power, 1927), has a small distribution range in northern South Africa and adjacent Botswana. Local populations have been fragmented by degradation and destruction of suitable habitat, resulting in this species being listed as Vulnerable by IUCN. Here, the geographic distribution of *K. lobatsiana* is updated and several hitherto unpublished occurrences are reported, which extend its distribution range to the north.

Keywords. Africa, chelonians, geographic distribution, Reptilia, South Africa, Testudinidae

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The Lobatse Hinge-back Tortoise, *Kinixys lobatsiana* (Power, 1927), is near-endemic to northern South Africa. The putative distribution range of this species covers an area of approximately 93,000 km² (extent of occurrence), which mainly falls within the South African province of Limpopo but also extends into the adjacent North West, Gauteng, and Mpumalanga provinces as well as into neighboring south-eastern Botswana (Fig. 1; Hofmeyr and Boycott 2018; Power 1927; TTWG 2017).

In the north, the distribution range was reported to reach to south of the Soutpansberg mountain range (Boycott 2014; Hofmeyr and Boycott 2018), with the sole specimen recorded further north assumed to represent a translocated individual (Boycott 2014; Broadley 1993; Hofmeyr and Boycott 2018; TTWG 2017). The Lobatse Hinge-back Tortoise was described as being closely associated with rocky hillsides and outcrops (Boycott 2014; Boycott and Bourquin 2000; Broadley 1989; Hofmeyr and Boycott 2018) and uses rock crevices and abandoned animal burrows as hiding places (Bonin et al. 2006; Broadley 1989). However, in the frame of the present study the species was also found in open savanna habitats, with only few rocks or burrows, using low shrubs (*Grewia* sp. and *Boscia albitrunca*) for shelter (Fig. 2).

In some of these sites many living tortoises examined had extensive burn marks on their shells, indicating that fires represent a significant threat. While this habitat type is naturally prone to burning, intentional fire as a management tool (e.g., set to stimulate new growth of nutritious vegetation) is often practiced on a higher frequency than natural fires would occur. Thus, higher abundances in rocky habitats might result from a higher survival rate due to the availability of sufficient shelter rather than a real habitat preference.

The species is considered to be the most arid-adapted *Kinixys* (Branch 2008) and was reported from various vegetation types across the central bushveld bioregion. These include dense, short bushveld and thornveld, open tree savannas, *Burkea* savannas, mixed thornveld, and *Combretum* woodlands (Boycott 2014; Branch 2008; Broadley 1989; Hofmeyr and Boycott 2018). *Kinixys lobatsiana* was reported to be absent from highveld grassland and subtropical lowveld (Boycott 2014; Branch 2008; Broadley 1989; Hofmeyr and Boycott 2018). However, we also found the species in a mosaic of highveld grassland and Loskop mountain bushveld in the Ezemvelo Nature Reserve. Degradation and destruction of suitable habitat have been identified as the main

Correspondence. *flora.ihlow@senckenberg.de, melita.vamberger@senckenberg.de, uwe.fritz@senckenberg.de, ryanvanhuyssteen@gmail.com, nycteris.cory2saint@gmail.com

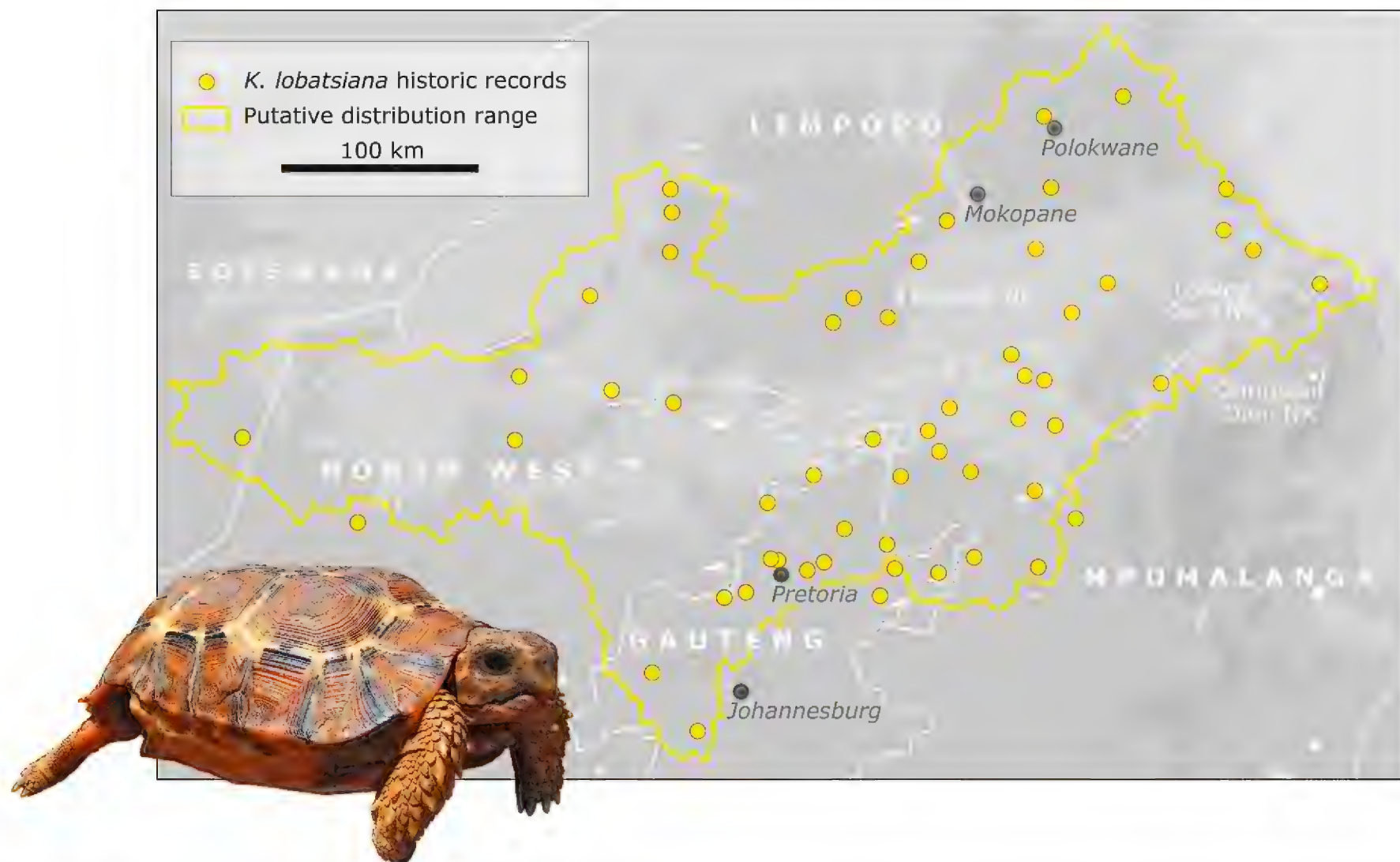


Fig. 1. Putative range of the Lobatse Hinge-back Tortoise (*Kinixys lobatsiana*) according to TTWG (2017), with historic records compiled from scientific collections. Inset: *K. lobatsiana* from the Lapalala Wilderness Reserve. Photo by Flora Ihlow.

threats for *K. lobatsiana*. While land use varies, habitat transformation is considered particularly severe in north-eastern South Africa and Botswana, where approximately 20–25% of suitable habitat has been destroyed within the last four decades, and a loss totalling 35–40% is expected within the next 30–40 years (Hofmeyr and Boycott 2018). Intact habitat appears to be fragmented and is largely restricted to protected areas and private reserves. Consequently, the IUCN conservation status of *K. lobatsiana* has recently been elevated from Least Concern to Vulnerable (Hofmeyr and Boycott 2018).

Here, the geographic distribution range of the species is discussed based on genetically verified records, and several occurrences are reported that considerably extend its known range. Field research was conducted across most of the South African distribution range of *K. lobatsiana* as well as at selected sites containing suitable habitat outside of its known range. Study sites were selected by geo-referencing collection sites of museum specimens mentioned by Broadley (1989, 1993) and other published records (TTWG 2017). The dataset was supplemented with selected observations (i.e., those where photographic vouchers allowed species identification) from the ReptileMap database (FitzPatrick Institute of African Ornithology 2019; <http://vmus.adu.org.za>). Searches for *K. lobatsiana* were carried out during the species' daily activity times and suitable weather conditions. GPS coordinates were collected for each individual. All tortoises were identified based on established morphological traits and coloration patterns (Broadley 1993), measured, and photographed from several angles.

In addition, a small (0.2 ml) blood sample was drawn from the subcarapacial sinus using a 1 ml syringe and a 25-G needle for genetic verification of species identification. Blood samples were either preserved using Whatman paper (GE Healthcare, Munich, Germany) or in analytical ethanol, and subsequently processed at the molecular genetic laboratory of the Museum of Zoology, Senckenberg Dresden, Germany. Species identification was genetically verified using mtDNA fragments containing the *cyt b* gene or the partial ND4 gene plus adjacent DNA coding for tRNAs following methods outlined in Kindler et al. (2012). European Nucleotide Archive (ENA) accession numbers of voucher sequences for extralimital records are listed in Table 1.

The following hitherto unreported occurrences extend the range of the species in two regions to the north, namely in the northern Waterberg region and the western Soutpansberg area (Fig. 2). Sampling sites in the Waterberg region include the Lapalala Wilderness Reserve, covered by a vegetation type classified as Waterberg mountain bushveld (Dayaram et al. 2017), and the private Kudu Canyon Reserve (central sandy bushveld; Dayaram et al. 2017) located in the vicinity of the Mokolo Dam Nature Reserve. Sites in the western Soutpansberg area comprise Sigurwana and the neighboring Goro Game Reserve (Soutpansberg mountain bushveld; Dayaram et al. 2017; Table 1). The Leshiba Wilderness and the Medike Nature Reserve neighboring Sigurwana in the east provide similar high-altitude habitat, but harbor *K. spekii* instead of *K. lobatsiana*. However, additional searches targeting high elevation habitat in the Medike Nature Reserve will

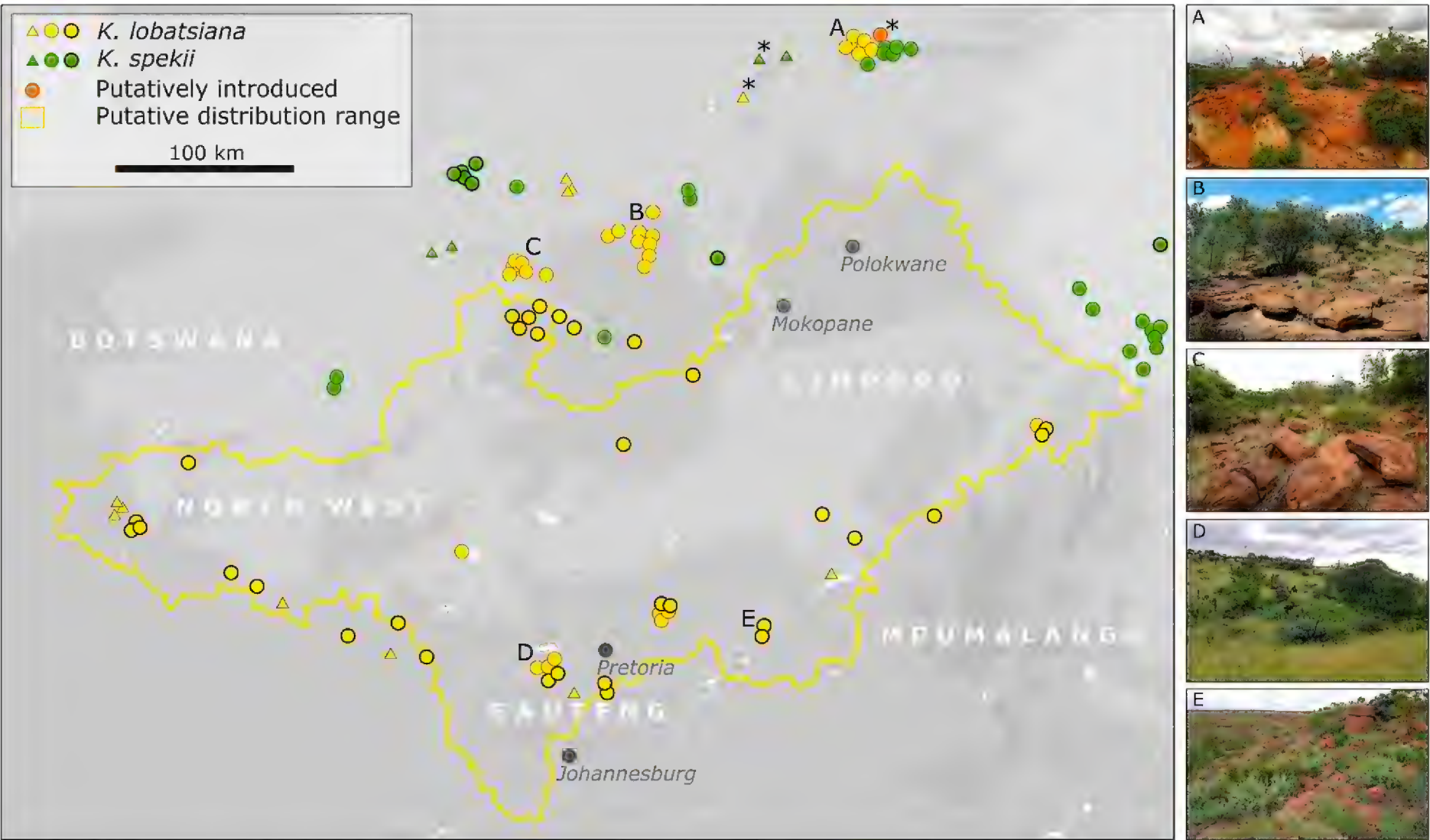


Fig. 2. Distribution range of the Lobatse Hinge-back Tortoise (*Kinixys lobatsiana*) according to TTWG (2017), with genetically confirmed range extensions (dots), not yet processed samples (dots with bold black outline), and recent observations (triangles). Observations marked with an asterisk refer to collection material in the Ditsong National Museum of Natural History (TM 36366, TM 67909, TM 79431). Right: Characteristic habitats from different parts of the distribution range. *Photos by Flora Ihlow.*

be required to fully clarify the distributions of *K. spekii* and *K. lobatsiana* in this area. The samples reported here, published records, and museum specimens consistently suggest that *K. spekii* also inhabits the surrounding lowlands (Schmidt 2002).

Only a few herpetological surveys have been previously conducted within the Soutpansberg Mountain Range (e.g., SARCA surveys contributing to Bates et al.

2014; Jacobsen 1989; Kirchhof et al. 2010). Most studies either targeted areas covered with habitat unsuitable for *K. lobatsiana* or focused on taxa with considerably different activity profiles. As a result, *K. lobatsiana* has not been recorded from the area thus far, except for a single record from north of the Soutpansberg mountains that was considered to refer to a misplaced individual (Broadley 1993; Hofmeyr and Boycott 2018; TTWG

Table 1. Records extending the distribution range of the Lobatse Hinge-back Tortoise (*Kinixys lobatsiana*) to the north. Coordinates obscured to protect populations against poaching. ENA = European Nucleotide Archive; MTD T = Museum of Zoology, Senckenberg Dresden (Tissue Collection).

MTD T	ENA number	Study site	Latitude	Longitude	Elevation
17056	LR746292	Lapalala Wilderness Reserve	-23.8	28.3	1,160 m
17062	LR746293	Lapalala Wilderness Reserve	-23.8	28.3	1,160 m
17064	LR746294	Lapalala Wilderness Reserve	-23.8	28.3	1,100 m
17067	LR746295	Lapalala Wilderness Reserve	-23.8	28.3	1,040 m
17071	LR746296	Lapalala Wilderness Reserve	-23.7	28.3	1,100 m
17076	LR746297	Lapalala Wilderness Reserve	-23.8	28.2	1,060 m
17078	LR746298	Lapalala Wilderness Reserve	-23.9	28.3	1,160 m
17079	LR746299	Lapalala Wilderness Reserve	-23.9	28.3	1,140 m
20164	LR746300	Kudu Canyon	-24.0	27.7	1,009 m
20169	LR746301	Kudu Canyon	-24.0	27.7	930 m
20176	LR746302	Goro Game Reserve	-22.9	29.4	1,086 m
20188	LR746303	Sigurwana	-22.9	29.4	1,380 m
20189	LR746304	Sigurwana	-22.9	29.4	1,360 m
20738	LR746305	Sigurwana	-22.9	29.4	1,380 m
20739	LR746306	Sigurwana	-22.9	29.4	1,440 m



Fig. 3. Lobatse Hinge-back Tortoise (*Kinixys lobatsiana*, TM 36366) collected at Rochdale Farm, Waterpoort, from the Ditsong National Museum of Natural History. Photo by Adriaan Jordaan.

2017). This record likely corresponds to a museum specimen housed at the Ditsong National Museum of Natural History (TM 36366) collected in 1964 by G. Newlands on Rochdale Farm, Waterpoort. Rochdale Farm lies on the northern slope of the Soutpansberg, just east of Waterpoort. A morphological examination of TM 36366 confirms that it represents *K. lobatsiana* (Fig. 3). Broadley (1993) suggested that this specimen might have been “swept through the gorge by the Sand River during a flood” to reach its collection site, while Boycott (2014) added translocation by humans as another possible explanation. Considering that *K. lobatsiana* is confirmed from two other properties on the northern slope of the Soutpansberg Mountains, 22 km away from the collection site of TM 36366, that record no longer appears to be an outlier. The present evidence rather suggests a continuous distribution of *K. lobatsiana* along the northern slope of the western Soutpansberg.

Schmidt et al. (2005) reported *K. spekii* from the Blouberg Nature Reserve. Unfortunately, this finding could not be re-examined because neither access to the reserve nor photographs of the tortoise could be obtained so far. However, a specimen in the Ditsong National Museum of Natural History (TM 67909) collected from a farm located approximately 6 km west of the Blouberg Nature Reserve (The Glen, Quarter Degree Square 2328Bb) morphologically resembles *K. spekii*. In addition, two photo vouchers (163852 and 167289), uploaded to the virtual museum database (<http://vmus.adu.org.za>) showing the same individual from the southern lowlands of the Blouberg Nature Reserve, also resemble *K. spekii*. However, another specimen from

the Ditsong National Museum of Natural History (TM 79431) collected at Bochum, Limpopo (QDS 2329AC), morphologically resembles *K. lobatsiana*. Thus, further research is needed to clarify the distributions of these two species in this area.

The present report shows that the distributions of *K. lobatsiana* and *K. spekii* are still incompletely known. This contradicts Branch et al. (1995), who concluded that the distribution of South African tortoises and terrapins is relatively well-documented. Misidentifications of these two morphologically challenging species and the application of an outdated taxonomy compromise the previous assessments of their distributions. *Kinixys lobatsiana* and *K. spekii* were lumped together with other species under “*K. belliana*” before Broadley (1989, 1993) separated these taxa.

While Broadley (1993) stated that for *K. lobatsiana* no evidence of sympatry with any other species exists, later authors considered *K. lobatsiana* to co-occur with *K. spekii* throughout its range (TTWG 2017). However, only a few areas (Waterberg region, western Soutpansberg) were found where both species occur in close proximity. Juveniles and old specimens of both species tend to lack the characteristic shell shape and color patterns used to distinguish between the two species (Broadley 1993), which leads to high misidentification rates, compromises range estimates, and has implications for conservation measures that are based on erroneous records and range estimates (Figs. 4–5; Ihlow et al. 2019).

The most reliable morphological trait to distinguish challenging adult specimens of *K. spekii* and *K. lobatsiana* is the serrated posterior carapace rim in the latter species



Fig. 4. Top: Juvenile Lobatse Hinge-back Tortoise (*Kinixys lobatsiana*, SCL = 81 mm) from Sigurwana, western Soutpansberg, Limpopo. Bottom: Young Speke's Hinge-back Tortoise (*K. spekii*, SCL = 106 mm) from Leshiba Wilderness, western Soutpansberg. Scale bar: 1 cm. Species identification of both tortoises was genetically confirmed. *Photos by Flora Ihlow.*

(Ihlow et al. 2019). However, this character does not always suffice in juveniles (Fig. 4). To enable later re-examination, photographs should document the dorsal, ventral, and lateral aspects of each tortoise. In addition, molecular genetic confirmation is recommended.

Branch et al. (1995) concluded that most South African chelonian species are adequately protected by existing reserves. However, he stressed that *K. lobatsiana* was only recorded in three major reserves, namely the Loskop Dam Nature Reserve (Mpumalanga), the Ohrigstad Dam Nature Reserve (Mpumalanga), and the Nylsvley Nature Reserve (Limpopo). The species could be confirmed from a photo voucher taken by an employee of the Loskop Dam Nature Reserve in 2017, but records from the other two reserves have to

be considered uncertain. Only a single possible record exists for the wider Ohrigstad area (Ditsong National Museum of Natural History, TM 21329, a specimen closely resembling *K. lobatsiana*, collected by P. van Tonder in 1944). However, the Ohrigstad Dam Nature Reserve itself is located well outside of the putative range of the species. In the Nylsvley Nature Reserve, “*K. b. belliana*” was reported from a single photographed tortoise (Jacobsen 1977), which was identified as *K. lobatsiana* by Broadley (1993), but Jacobsen (2008) referred to the same record later as “*K. belliana spekei* [sic]”, suggesting that *K. spekii*, and not *K. lobatsiana*, occurs in the Nylsvley Nature Reserve. Unfortunately, no hinge-back tortoise has been recorded there for many years by the local rangers (pers. comm.) nor are there any



Fig. 5. Speke's Hinge-back Tortoise (*Kinixys spekii*) from the vicinity of Vaalwater (left) and Lobatse Hinge-back Tortoise (*K. lobatsiana*) from the Lapalala Wilderness Reserve (right) with very similar color patterns. Species identification of both tortoises was genetically confirmed. *Photos by Flora Ihlow.*

photographs of hinge-back tortoises from this reserve in any of the databases that were queried. Branch et al. (1995) further highlighted the Marakele National Park (former Kransberg National Park) and the Blyde River Canyon Nature Reserve as important statutory protected areas where the species has been recorded. However, we are not aware of any contemporary records from either of these reserves. In addition to these published localities, the occurrence of *K. lobatsiana* from the following sites was confirmed:

Gauteng: Ezemvelo Nature Reserve (Bronkhorst-spruit), Irene, Kalkheuwel, Leopard Lodge (Thiane Wildlife Sanctuary), Monateng Safari Lodge (East Lynne).

Limpopo: Bushfellows Lodge (Marble Hall), Goro Game Reserve (Western Soutpansberg), Inkwe Private Nature Reserve (Waterberg), Kalkfontein Farm (Groblersdal), Kudu Canyon Farm (Lephalale), Lapalala Wilderness Reserve (Waterberg), Lépelle Lodge (Burgersfort), Sigurwana Lodge (Western Soutpansberg), Thandabantu Game Lodge (Roos-senekal), Welgevonden Nature Reserve (Waterberg).

Mpumalanga: Loskop Dam Nature Reserve.

North West: Kgaswane Nature Reserve (Rustenburg), Koster (Rustenburg), land owned by the Bahurutshe Ba Ga Lencoe Traditional Council (Moshana village), Riekersdam Farm (Ramotshere Moiloa), Tswenyane Safaris (Zeerust), Vaalkop Dam Nature Reserve (Rustenburg).

While the species appears to be rare in most areas, larger populations were recorded in the Waterberg area (Lapalala Wilderness Reserve, the Welgevonden Nature Reserve, and in the vicinity of the Mokolo Dam Nature Reserve), in the vicinity of Kalkheuwel, and in the vicinity of Lobatse, Botswana.

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NW6124/10/2018, and Gauteng: CPF6-0210. Field research was partly funded through the Mapula Trust awarded to Margaretha D. Hofmeyr. Flora Ihlow profited from a Margarethe Koenig Scholarship of the Zoological Research Museum Alexander Koenig and is currently supported by the German Science Foundation (DFG IH 133/1-1).

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Flora Ihlow is a German herpetologist (Dr. rer. nat.) presently working at the Senckenberg Natural History Collections, Dresden, Germany. For the past 10 years, Flora's research predominantly focused on the ecology, systematics, and distribution of Southeast Asian chelonians. She has published numerous scientific papers on the herpetofauna of Southeast Asia. After her graduation from the Rheinische Friedrich Wilhelms University (Bonn, Germany), Flora joined the phylogeography department of Senckenberg, Dresden in 2017 to work on the systematics and distribution of chelonians from southern Africa within the frame of a post-doctoral position. Flora is a member of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group (TFTSG).



Ryan Van Huyssteen is a South African field biologist interested in reptile distribution, biogeography, and ecology. Ryan currently lives in the Soutpansberg, Limpopo, South Africa, where he has been working on reptiles and conservation for the past five years.



Melita Vamberger is a Slovenian herpetologist and evolutionary biologist working at the Senckenberg Natural History Collections, Dresden, Germany. Melita studied Biology at the University of Ljubljana, Slovenia, focusing on the natural history of the European Pond Turtle (*Emys orbicularis*). After her diploma, Melita moved to Germany for her Ph.D. at the University of Leipzig, studying the phylogeography and hybridization of two closely related freshwater turtle species (*Mauremys capsica* and *M. rivulata*). Melita's main interests are speciation, gene flow, adaptation, and evolution of different turtle taxa using an integrative approach that combines genetic and ecological methods, especially in the Western Palearctic and sub-Saharan Africa.



Dawn Cory-Toussaint is currently a final year Ph.D. student at the University of Venda (South Africa) studying the ecological impacts of opencast diamond mining on bats and the role that bats can play as biological indicators in Limpopo. Dawn has a broad understanding and an unlimited interest in the natural world. She has been involved in projects on diverse organisms ranging from Killer Whales (satellite tagging and biopsy sampling) to beetles (Darkling Beetles of the Bushveld). Being involved in projects that are broader than her current study, particularly in northern Limpopo, is invaluable to her role in the management and conservation in the area where she currently resides and works.



Margaretha D. Hofmeyr was Professor Emerita at the Biodiversity and Conservation Biology Department, University of the Western Cape, South Africa. She was an ecophysiological by training and first studied large ungulates before switching to chelonians. Her ecophysiological studies revealed that South African tortoises have many unique characteristics, which stimulated her interest in their genetic diversity and systematics. Margaretha published extensively on the ecology and phylogeography of sub-Saharan tortoises and turtles, and she was closely involved in conservation projects on threatened tortoises. This work resulted in her being awarded the 2015 Sabin Turtle Conservation Prize. She was a member, and Regional Vice-Chair for Africa, of the IUCN/SSC TFTSG and coordinated the 2014 and 2018 Red List Assessment for South African tortoises and freshwater turtles.



Uwe Fritz is the head of the Museum of Zoology, Senckenberg Natural History Collections in Dresden, Germany, and Extraordinary Professor for Zoology at the University of Leipzig, Germany. Uwe has worked for many years on the taxonomy, systematics, and phylogeography of turtles and tortoises, and has also studied to a lesser extent snakes and lizards. He is particularly interested in hybridization patterns and gene flow in contact zones of distinct taxa. Uwe has authored or co-authored numerous scientific articles, mainly in herpetology, and has also edited proceedings and books, among them the two turtle volumes of the *Handbook of Amphibians and Reptiles of Europe*.



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A new species of *Petracola* (Squamata: Gymnophthalmidae) from Río Abiseo National Park, San Martín, Peru

^{1,*}Lily O. Rodríguez and ^{2,3,4}Luis Mamani

¹Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Lima, PERÚ ²Programa de Magister en Ciencias con mención en Zoología, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario S/N, Casilla 160C, Concepción, CHILE ³Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC), Plaza de Armas s/n (Paraninfo Universitario), Cusco, PERÚ ⁴Museo de Biodiversidad del Perú (MUBI), Urbanización Mariscal Gamarra A-61, Zona 2, Cusco, PERÚ

Abstract.—A new species of *Petracola* is described from Río Abiseo National Park in northeastern Peru, where it inhabits grasslands above montane forest at 3,230 m asl. The new species is diagnosed by a unique combination of morphometric, scalation, and color pattern characteristics, increasing the number of described *Petracola* species to five.

Keywords. Andean lizard, Cercosaurini, Gran Pajatén, Huallaga basin, montane forest, Reptilia

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Introduction

The genus *Petracola* Doan and Castoe 2005 is a lineage of semifossorial lizards distributed in the central and northern Andes of Peru (Doan and Castoe 2005; Echevarria and Venegas 2015; Kizirian et al. 2008; Köhler and Lehr 2004; Uzzell 1970). These small lizards occur in cloud forest, montane forest, and wet puna from 1,889 m to 3,600 m asl (Echevarria and Venegas 2015; Kizirian et al. 2008; Köhler and Lehr 2004).

The genus *Petracola* was created to resolve the polyphyly of *Proctoporus* (Doan and Castoe 2005) and was nested in Cercosaurinae as sister lineage of a lineage formed by *Cercosaura*, *Dendrosauridion*, *Potamites*, *Proctoporus*, *Selvasaura*, and *Wilsonosaura* (Torres-Carvajal et al. 2016; Moravec et al. 2018; Vasquez-Restrepo et al. 2019). It currently includes four species: *P. angustisoma* Echevarria and Venegas 2015, *P. waka* Kizirian, Bayefsky-Anand, Eriksson, Le, and Donnelly 2008, *P. labioocularis* (Köhler and Lehr 2004), and *P. ventrimaculatus* (Boulenger 1900). Compared to its close relatives, *P. ventrimaculatus* has a relatively extensive geographic distribution, including both sides of the Marañón River (Kizirian et al. 2008); furthermore, osteological, morphological, and ecological evidence suggest it could be a complex of species (Echevarria 2014).

In 1987, during a faunal inventory expedition to the upper part of the Río Abiseo National Park, Perú, the first author (LOR) collected five specimens with characteristics matching the *Petracola ventrimaculatus* group and deposited them in the herpetology collection of Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM). In this paper, this new species of *Petracola* is described based on morphological data, increasing the number of species to five.

Materials and Methods

Specimens and ecological data were collected in Río Abiseo National Park in 1987 by the first author (LOR). Specimens were collected by hand, euthanized, fixed in 10% formalin, and later transferred to 70% ethanol. Terminology for diagnostics and descriptions follows Kizirian (1996) and Goicoechea et al. (2013). Measurements were taken with calipers accurate to 0.1 mm. Data for other species were taken from the literature (Echevarria and Venegas 2015; Kizirian et al. 2008; Köhler and Lehr 2004) and by examination of specimens deposited in the Museo de Historia Natural de la Universidad Nacional de San Marcos (MUSM), Lima, Peru, and the Museo de Biodiversidad del Perú (MUBI), Cusco, Peru (Appendix I).

Correspondence. *lrodriguez@cima.org.pe



Fig. 1. Holotype of *Petracola pajatensis* **sp. nov.** (MUSM 3829; male, SVL = 60.5, Tail = 76.8 mm, scale bar = 10 mm).

Results

Generic Assignment

The new species is assigned to *Petracola* based on the presence of imbricate and scale-like papillae on the tongue, head scales without striations or rugosities, smooth and juxtaposed dorsal scales, and the absence of prefrontal scales (Doan and Castoe 2005).

Taxonomy

Petracola pajatensis **sp. nov.**

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Holotype. MUSM 3829, adult male (Figs. 1–2) from Los Chochos, Rio Abiseo National Park, Provincia Mariscal Cáceres, Departamento San Martín, Peru, approximately 3,230 m asl, 18 km airline from Pataz (7°38'13"S, 77°28'80"W), collected by Lily O. Rodriguez on 12 July 1987.

Paratypes. Three adult females (MUSM 3830 [Fig. 2], 15986–87), and one subadult male (MUSM 15985), same data as holotype.

Diagnosis. (1) Frontonasal and frontal scales sub-equal; (2) nasoloreal suture present, loreal scale not in contact with supralabials; (3) supraoculars two; (4) superciliaries two, discontinuous, first expanded onto dorsal surface of head; (5) postoculars two; (6) palpebral disc divided in two; (7) three supralabials anterior to the posteroventral angle of subocular; (8) two pairs of genials in contact;

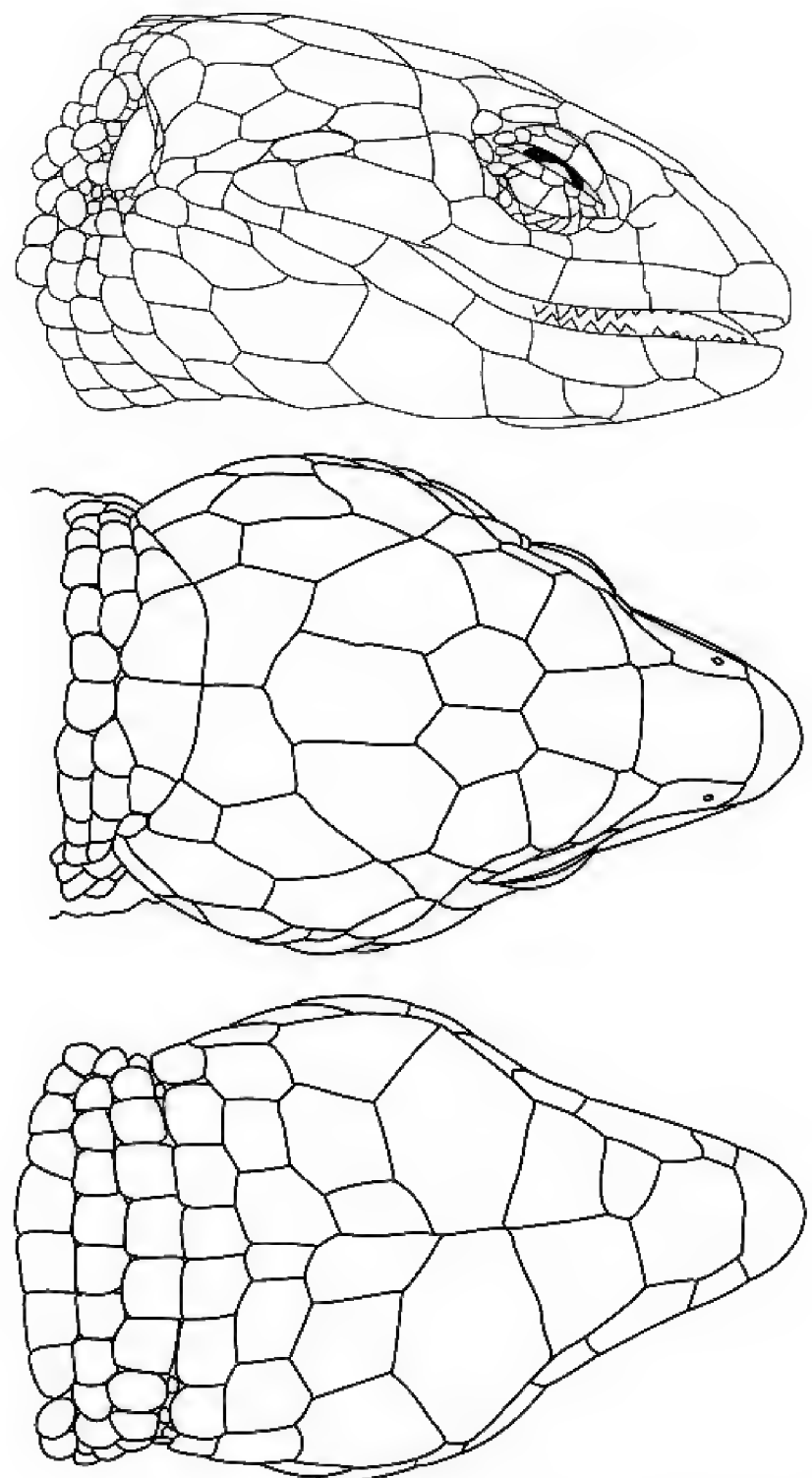


Fig. 2. Dorsal, lateral, and ventral views of head of the holotype (MUSM 3829).



Fig. 3. Dorsal and ventral views of the paratype of *Petracola pajatensis* **sp. nov.** (MUSM 3830; female, SVL = 65.1, Tail = 64.3 mm).

(9) dorsal body scales quadrangular, smooth, juxtaposed; (10) transverse dorsal rows 33–35; (11) transversal ventral rows 20–22; (12) a continuous series of small lateral scales separates dorsals from ventrals; (13) posterior cloacal plate scales 2–5; (14) anterior preanal plate scales paired; (15) femoral pores per hind limb in males 6–8, in females three; (16) preanal pores absent; (17) subdigital lamellae on finger IV eight; (18) limbs not overlapping when adpressed against body on adults; (19) pentadactyl, digits clawed; (20) coloration in liquid preservative: in males, dorsum is light-brown with numerous irregular dark-brown spots and venter is dark-brown with some small cream spots on their flanks; in females, dorsum is light-brown with some and irregular dark-brown spots, venter is brown with cream spots that form discontinuous transversal bands from the chest to the anal plate (Fig. 3).

Petracola pajatensis **sp. nov.** can be distinguished from *P. angustisoma* by having a robust body, two discontinuous superciliaries, 6–8 femoral pores per hind limb in males, maximum SVL in males 60.5 mm, dorsum is light-brown with irregular dark brown spots not forming longitudinal stripes, venter is brown in preservative (gracile body, three discontinuous superciliaries, nine femoral pores per hind limb in males, maximum SVL in males 43.6 mm, dorsum is brown or olive with seven discontinuous dark brown longitudinal stripes, venter is white with black semicircular black spots on anterior margin of scales); from *P. labioocularis* by having two supraoculars, absence of precloacal pores, and two pairs of genials in contact (three supraoculars, presence of precloacal pores, and usually three pairs of genials in contact); from *P. ventrimaculatus* by having 6–8 femoral pores in males, in preservative the venter

in males is dark brown with small lateral cream spots, in females it is a combination of cream with brown forming longitudinal bands, and maximum SVL in males 60 mm (2–5 femoral pores in males, in males and females the venter is cream with a bold black transversal band, and maximum SVL in males 71.05 mm); from *P. waka* by having two discontinuous superciliaries, two genials in contact, and venter in males is a dark brown with lateral cream spots, in females the venter is a combination of cream with brown forming a longitudinal band (four continuous superciliaries, three genials in contact, and the venter in males is white to pale yellow with brown spots).

Description of the holotype. Adult male, snout-vent length (SVL) 60.5 mm, tail length 76.8 mm, head scales smooth, rounded in lateral and ventral views, without striations or rugosities; rostral scale wider than tall, in contact with frontonasal, nasals, and first supralabials; frontonasal longer than wide, longer than the frontal scale, widest posteriorly, in contact with rostral, nasal, first superciliary, and frontal; prefrontal absent; frontal longer than wide, pentagonal, in contact with first superciliary, first supraocular, and frontoparietal; frontoparietal paired, polygonal (hexagonal), in contact with frontal, supraoculars, parietals, and interparietals; supraoculars two, the first separates the first and second superciliaries, in contact with superciliaries, frontal, frontoparietals, interparietal, and postoculars; parietals longer than wide, polygonal (irregular hexagon), in contact with frontoparietals and supraoculars anteriorly, with interparietal, and temporals laterally, and with postparietals posteriorly; interparietal polygonal (hexagonal), in contact with frontoparietals anteriorly, with parietals laterally, and with postparietal



Fig. 4. Dorsolateral view of unvouchered living specimen from Los Chochos. *Photo by Ken R. Young.*

posteriorly; postparietals paired, smaller than parietals, and polygonal. Nasal scale divided, longer than high, in contact with first supralabials; loreal scale present on the left side, nasoloreal suture incomplete on the right side, not in contact with the supralabials; two superciliaries, discontinuous, and first expanded onto the dorsal surface of the head; two preoculars; frenocular fused with the first subocular only on the right side; palpebral disc transparent and divided in two; three suboculars, on the right side the first subocular is fused with the frenocular; two postoculars; temporals smooth, polygonal; three supralabials anterior to posteroventral angle of suboculars. Mental wider than long, in contact with first infralabials and postmental posteriorly; postmental single, polygonal (irregular heptagonal), in contact with the first and second infralabials, and the first pair of genials; 3/2 genials, on right and left sides respectively, all in contact medially, the first genial on right side is divided; three transversal rows of preangular scales; six gular scale rows, polygonal, and smooth. Dorsal scales rectangular, longer than wide, juxtaposed, smooth, 33 transverse rows; 21 longitudinal dorsal scale rows at midbody; a continuous series of small lateral scales; reduced scales at limb insertion region present; 21 transverse ventral scale rows; 10 longitudinal ventral scale rows at midbody, the lateral scales are slightly smaller; anterior preanal plate scales paired; three posterior preanal plate scales, and a small and polygonal scale lies between the anterior and posterior preanal plate scales; scales on the tail rectangular, juxtaposed, and smooth; ventral scales quadrangular, juxtaposed, and smooth. Limbs pentadactyl; digits clawed; dorsal brachial scales polygonal, subimbricate, and smooth; ventral brachial scales rounded, subimbricate, and smooth; dorsal antebrachial scales polygonal, smooth; ventral antebrachial scales polygonal, smooth, smaller than dorsal; dorsal manus scales polygonal, smooth, subimbricate; palmar scales small, rounded, juxtaposed, and domelike; dorsal scales on fingers smooth, quad-

angular, imbricate, three on finger I, five on II, six on III, five on IV, and four on V; scales on anterodorsal surface of thigh polygonal, smooth, subimbricate; scales on posterior surface of thigh small, rounded, and juxtaposed; scales on ventral surface of thigh small, polygonal and juxtaposed; six femoral pores on left thigh and seven on right; scales on anterior surface of crus polygonal, smooth, juxtaposed, decreasing in size distally; scales on posterodorsal surface of crus smooth, polygonal, juxtaposed; scales on ventral surface of crus polygonal, enlarged, smooth, and subimbricate; scales on dorsal surface of toes polygonal, smooth, and subimbricate; scales on ventral surface of toes rounded, small, and domelike; dorsal scales of toes smooth, imbricate, two on toe I, five on toe II, seven on toe III, ten on toe IV, and six on toe V.

Coloration

In preservative. *Petracola pajatensis* **sp. nov.** exhibits a variable coloration in adults of both sexes. The males (Fig. 1) have a light-brown dorsum with numerous and irregular dark-brown spots, with a cream continuous dorsolateral line on both sides of the body that starts from the back of the eye to the tail. This cream line is bordered on both sides by a continuous dark-brown line; the venter is dark-brown with some small cream spots on their flanks. The females (Fig. 3) have a light-brown dorsum with some irregular dark-brown spots, with a cream discontinuous dorsolateral line on both sides of the body that starts from the back of the eye to the tail; the venter is brown with cream spots that form continuous longitudinal bands from the chest to the anal plate; the neck and chin are a combination of cream and brown.

In life. Based on unvouchered specimens, the dorsal coloration is brown with irregular dark spots distributed irregularly, the dorsolateral lines are obscure, and the flanks have more dark spots than the dorsum (Fig. 4).

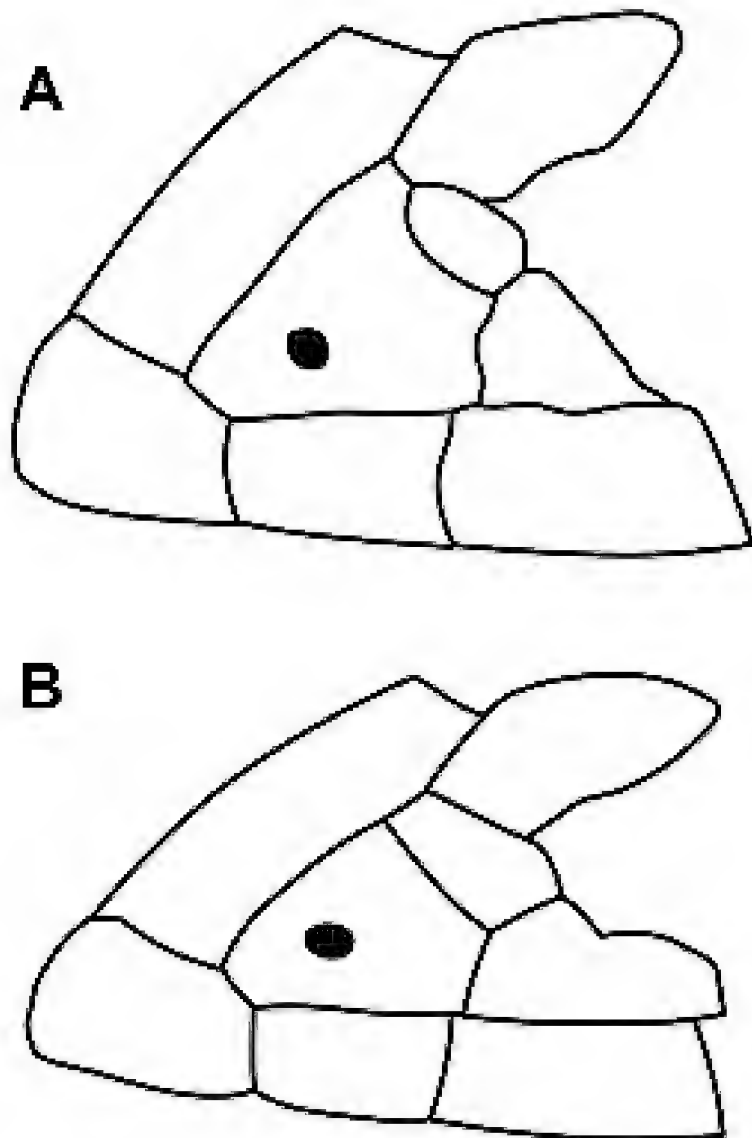


Fig. 5. Drawings of the right side showing the condition of loreal scales in *Petracola pajatensis* **sp. nov.** A) MUSM 3829, B) MUSM 3830.

Variation

Considerable variation is evident in the position and form of the loreal scale. The loreal scale can be incomplete, tiny, or enlarged; and can be separating, or not, the first superciliary and nasal scale; in the holotype (MUSM 3829) the right scale is incomplete and the left does not separate the nasal and first superciliary, while in three paratypes (MUSM 3830, 15985–6) the loreal scale separated the nasal and first superciliary; and in one paratype (MUSM 15987) the first superciliary and nasal are in contact on both sides (Figs. 2, 5). Variation in meristic characters is shown in Table 1. No evidence of sexual dimorphism exists in the scutellation, except for the larger number of and better developed femoral pores in males.

Etymology

The specific epithet is an adjective that recognizes Gran Pajatén archaeological remains, which, like *Petracola pajatensis* **sp. nov.**, occurs in Río Abiseo National Park.

Habitat, Ecological Notes, and Distribution

The holotype was taken from the ground in grassland, in a rocky area dominated by bunchgrasses of *Calamagrostis*



Fig. 6. Type locality of *Petracola pajatensis* **sp. nov.** Photo by Ken R. Young.

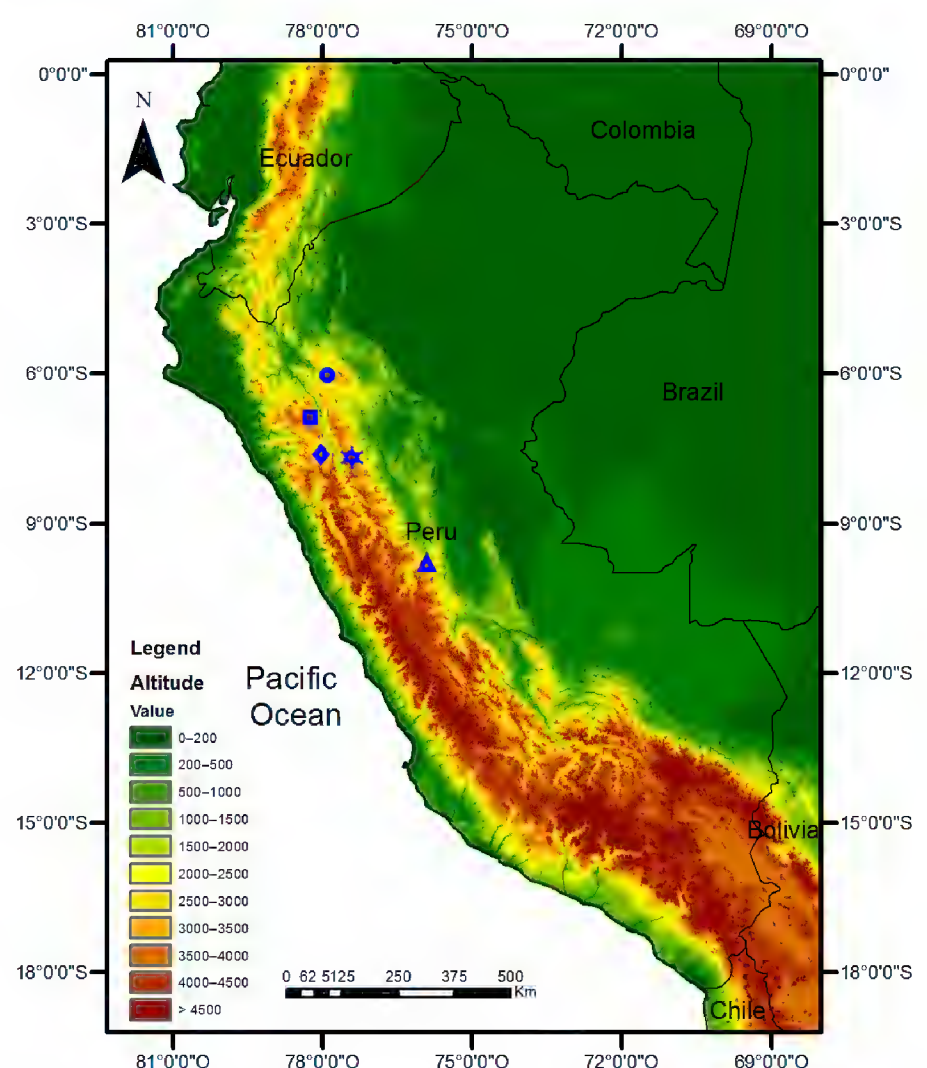


Fig. 7. Locations of the type localities of the species of *Petracola* (*P. angustisoma* = circle, *P. ventrimaculatus* = square, *P. waka* = diamond, *P. labioocularis* = triangle, *P. pajatensis* **sp. nov.** = star). Sources: Echevarría and Venegas (2015), Echevarría (2014), Kizirian et al. (2008), and Köhler and Lehr (2004).

sp., *Festuca* *sp.*, *Cortaderia* *sp.*, and *Stipa* *sp.* (Fig. 6). The habitat of *Petracola pajatensis* **sp. nov.** was previously disturbed through overgrazing and is currently undergoing secondary succession, including scattered shrubs (*Baccharis*). Multiple nest deposition sites contained elliptical egg shells (9.5–11.0 mm). The new species is known only from Los Chochos, a small valley on the western slopes of the Cordillera Oriental, south of the Huancabamba Depression, approximately 100 km south of the localities of *P. ventrimaculatus* (Fig. 7).

Table 1. Measurements and scale counts of specimens of *Petracola pajatensis* sp. nov. and *P. ventrimaculatus* examined in this paper. *only an adult male, ** the first right genial is divided, ***the anterior subocular scale is fused with freno ocular.

Characters (count or mm)	<i>Petracola pajatensis</i> sp. nov.		<i>Petracola ventrimaculatus</i>	
	Male (<i>n</i> = 2)*	Female (<i>n</i> = 3)	Male (<i>n</i> = 3)	Female (<i>n</i> = 2)
Snout vent length	60.5	52.8–65.1	60.8–71.05	48.2–59.0
Head length to ear	13.6	10.2–12.5	12.2–14.9	9.6–11
Head width	9.9	6.7–7.7	9.9–11	6.5–7.4
Supraoculars	2.0	2.0	2.0	2.0
Genials in contact	4–5**	4.0	4.0	4.0
Postoculars	2.0	2.0	2.0	2.0
Superciliaries	1–2, discontinuous	2, discontinuous	2–4, discontinuous	2–3, discontinuous
Supralabials	5.0	5–6	7.0	7.0
Supralabial scales to posteroventral angle of subocular	3.0	3.0	3.0	3–4
Subocular	3***	3.0	2–3	3–4
Infralabials	5–6	5–6	6.0	6.0
Postparietals (occipitals)	2.0	2.0	3–4	3.0
Temporals	11–15	12–15	12–18	13–14
Pregular scale rows	3.0	3.0	3.0	3.0
Gular scale rows	6.0	5–6	6.0	6.0
Scales around midbody	17–21	28–34	33–34	32–35
Longitudinal dorsal scale rows	17–21	16–20	22–23	20–23
Longitudinal ventral scale rows	8.0	8.0	10.0	10.0
Transversal dorsal scale rows	33–34	34–35	32–34	31–34
Transversal ventral scale rows	21.0	20–22	21–23	20–21
Femoral pores	6–8	2–3	4.0	3–4
Lamellae under 4 th finger	8–10	8–9	9–10	9–10
Lamellae under 4 th toe	14–15	13–14	12–13	14–15

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Lily O. Rodríguez is a herpetologist, holding a doctoral degree in Ecology from the Université de Paris. With extensive fieldwork in the Peruvian Amazon, Lily has a special interest in the diversity and distribution of herpetofauna across Peru. She is currently working for the Center for Conservation, Research, and Management of Natural Areas, CIMA-Cordillera Azul and is a Research Associate to the Museo de Historia Natural of the Universidad Nacional Mayor de San Marcos in Lima, Peru.



Luis Mamani is a biologist who graduated from the Universidad Nacional de San Antonio Abad del Cusco (Peru) and he obtained his M.Sc. degree from the Universidad de Concepción (UdeC) in Chile. Currently, Luis is a researcher at Museo de Biodiversidad del Perú (MUBI) and Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC). His current research includes systematics, taxonomy, and biogeography of the gymnophthalmid lizards from the Cordillera de los Andes.

Appendix 1. Specimens examined.

Petracola waka

PERU: Cajamarca: Baños del Inca: MUBI 4721, 4722, 4723; Cajabamba: MUBI 2609–2619, KU 135063–79; Celendin: El Sauce: MUSM 26246–26248, 26346–26351.

Petracola ventrimaculatus

PERU: Cajamarca: Celendín: MUBI 11114, 11117, 1118; Hualgayoc: MUBI 11402, 11403.

Petracola labioocularis

PERU: Huanuco: Monte Potrero: MUBI 14252, 14253, 14254, 14232, 14251; Acomayo: MUSM 13903, 13904.

Petracola angustisoma

PERU: Amazonas: Bongara: San Carlos: Achupampa: MUBI 11513.



The herpetofauna of Honaz Mountain National Park (Denizli Province, Turkey) and threatening factors

^{1,*}Müge Gidiş and ²Eyup Başkale

¹Kütahya Dumlupınar University, Faculty of Arts and Science, Department of Biochemistry, Kütahya, TURKEY ²Pamukkale University, Faculty of Arts and Science, Department of Biology, Denizli, TURKEY

Abstract.—The aim of this study was to catalog the species of amphibians and reptiles in Honaz District, in the province of Denizli, Turkey, based on field surveys carried out during March–October of 2015–2018. The species found include five amphibians (*Bufo bufo*, *Bufo variabilis*, *Pelophylax bedriagae*, *Hyla orientalis*, *Rana macrocnemis*) and 22 reptiles: two turtles (*Mauremys rivulata*, *Testudo graeca*), nine lizards (*Stellagama stellio*, *Mediodactylus kotschy*, *Anatololacerta danfordi*, *Lacerta trilineata*, *Ophisops elegans*, *Ablepharus kitaibellii*, *Pseudopodus apodus*, *Blanus strauchi*, *Heremites auratus*), and 11 snakes (*Xerotyphlops vermicularis*, *Dolichophis jugularis*, *D. caspius*, *Hemorrhois numnifer*, *Eirenis modestus*, *Telescopus fallax*, *Elaphe sauromates*, *Natrix natrix*, *N. tessellata*, *Eryx jaculus*, *Montivipera xanthina*). In addition, the various factors threatening these species in this area were determined and proposals for the conservation of these species are presented.

Keywords. Amphibians, Anura, distribution, habitat destruction, pollution, reptiles, Squamata, Testudines

Citation: Gidiş M, Başkale E. 2020. The herpetofauna of Honaz Mountain National Park (Denizli Province, Turkey) and threatening factors. *Amphibian & Reptile Conservation* 14(1) [General Section]: 147–155 (e228).

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Introduction

Amphibians and reptiles are an important component of most ecosystems. In particular, their importance is incontrovertible for the maintenance of food networks. Their prey includes a variety of insects and their larvae, fish, shellfish, other reptiles, other amphibians, birds, and small mammals. They are also a source of food for fish, birds, reptiles, and mammals. From this point of view, amphibians and reptiles play an important role in ensuring the balance within ecosystems (Böhm et al. 2013; Heatwole and Wilkinson 2015). In addition, because they are poikilotherms and as they are in close contact with the air, water, and soil according to the characteristics of the habitats in which they are located, they have good characteristics as indicators for food networks (Duellman and Trueb 1994) and environmental changes (Demirsoy 1996; Baran and Atatür 1998; Budak and Göçmen 2008; Baran et al. 2012). In recent years, studies focusing on the assessment of species diversity among ecological communities have been instrumental in developing protection strategies, as well as helping to clarify the structure and dynamics of biological communities (Baldwin et al. 2006; Shaney and Marshall 2013; Berriozabal-Islas et al. 2017; Ćurić et al. 2018).

Amphibians and reptiles are two of the most endangered groups compared to other vertebrates (Hoffmann et al. 2010). The herpetofauna of Turkey has been studied intensively in the last 50 years, providing information about the distribution of the many amphibian and reptile species in Turkey (Sillero et al. 2014; Yıldız and İğci 2015; Bülbül et al. 2016; Göçmen and Karış 2017; Sarıkaya et al. 2017; Gül et al. 2018; Yıldız et al. 2019). However, due to technological and methodological developments the taxonomy of reptiles and amphibians has remained dynamic and unstable. For example, in 2008, only 8,734 reptile species were reported for the world, but as of July 2018 this figure had increased to 10,793 (Reptile Database: <http://www.reptile-database.org>). Amphibian species are represented by about 7,950 species globally (AmphibiaWeb: <http://www.amphibiaweb.org>), and from 2004 through November 2018, a total of 2,318 new amphibian species were described, including 139 from January 2018 until 14 November 2018. A similar situation is found in Turkey, where the number of known amphibian and reptile species was 141 in 2013, and with the latest developments, the number of species has grown to 170 (Sindaco et al. 2000; Yıldız and İğci 2015; Bülbül et al. 2016; Yıldız et al. 2019).

Correspondence. *mugegidis@gmail.com

The history of studies on the Turkish herpetofauna traces back to the 19th century (Boettger 1889), and in recent years many new species have been identified and new records have been published (Sillero et al. 2014; Yıldız and İçci 2015; Bülbül et al. 2016; Wielstra and Arntzen 2016; Göçmen and Karış 2017; Sarıkaya et al. 2017; Gül et al. 2018; Yıldız et al. 2019). Faunistic inventory studies in Denizli province were conducted in 2013 by the Ministry of Forestry and Water Affairs, General Directorate of GDMP, and the Pamukkale University Biology Department. However, that study did not provide much information about the species and the factors that threaten them in the province of Denizli. The current survey (conducted from 2015–2018) focused on the herpetological species of the Honaz Mountain National Park-Denizli, which is one of Turkey's most important protected areas, and elucidating the threatening factors for amphibian and reptile species living in this national park. The results provide a new opportunity for revising the conservation status of the Honaz Mountain National Park-Denizli herpetofauna and a new reference for distributional data.

Materials and Methods

Honaz Mountain National Park-Denizli, which is located within the borders of the Honaz district of Denizli province, was declared a National Park on 21 April 1995 and approved by the General Directorate of Nature Conservation National Parks on 9 March 2009. The area of the national park is 9,616 ha and the highest point has an altitude of 2,571 m. The climate is dominated by the Mediterranean climate and the park is mostly covered with Red Pine (*Pinus brutia*) forest. A map of the Honaz Mountain National Park and the survey sites is given in Fig. 1.

Field surveys were conducted between March and October in each year from 2015 to 2018. Amphibians and reptiles in this area are active during spring and summer, which is the best time to study them. Early autumn can also be considered suitable for observing reptiles before hibernation. The Visual Encounter Survey (VES, Crump and Scott 1994) method was used in the field studies to assess the amphibian and reptile species. In this study, two teams conducted the field studies along linear paths according to the habitat characteristics, such as forest land, stony areas, and bush. The specimens captured during the surveys were diagnosed, and assessed at the species or family levels. According to the size of the habitat, 0.5–1.5 h of fieldwork was performed at each station. Specimens which could not be identified in the field were transported to the laboratory, and species were diagnosed by using an identification key or the current literature for the relevant species (Baran and Atatür 1998; Özeti and Yılmaz 1994; Baran et al. 2012; Budak and Göçmen 2008).

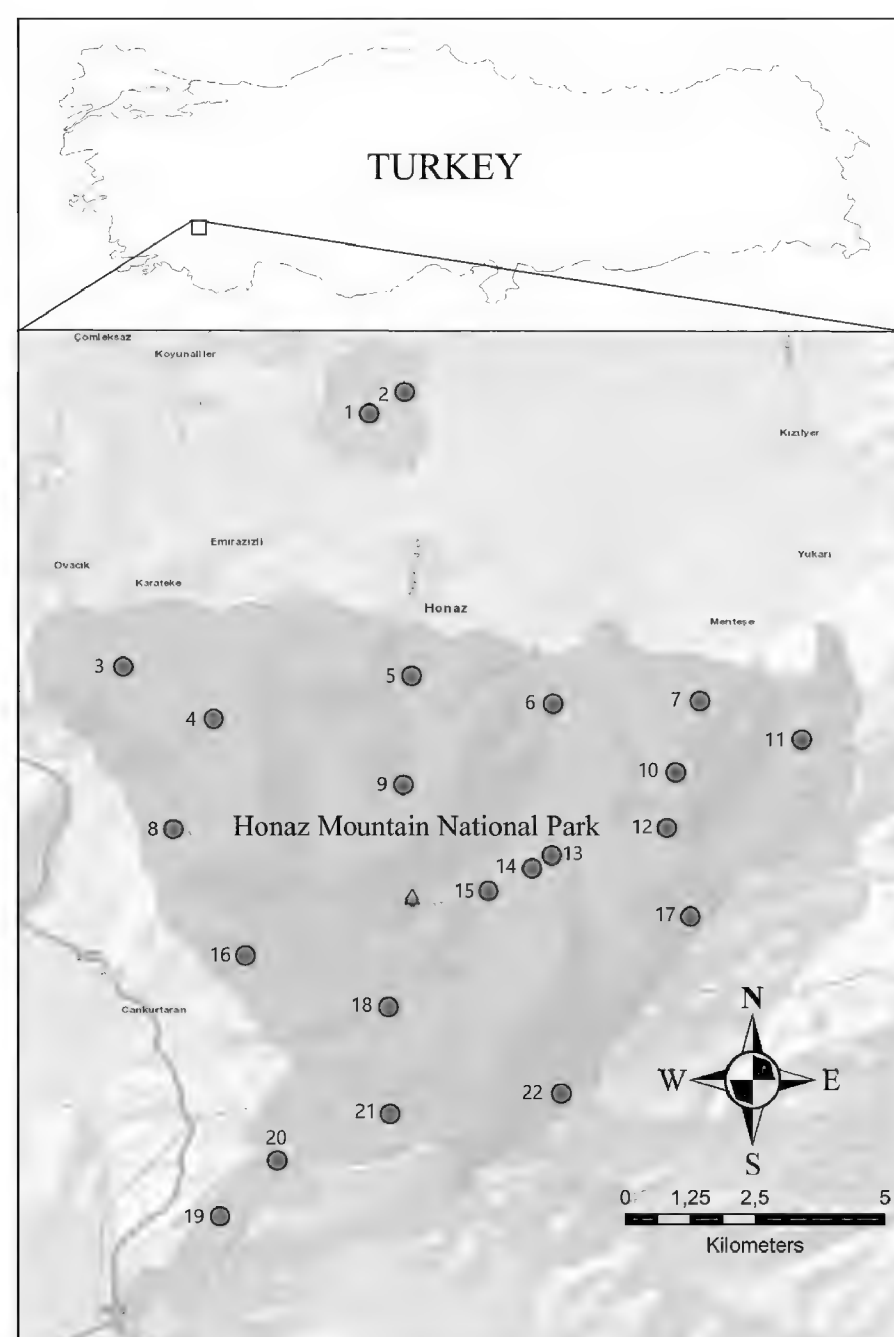


Fig. 1. Study area and locations of field surveys in Honaz Mountain National Park, Turkey. The coordinates and elevations of the numbered sites are given in Appendix 1.

After the species were identified, their population status was further determined using the following resources: 1) Convention on the Conservation of European Wildlife and Natural Habitats = BERN (Bern 1982), 2) Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 1975), and 3) The International Union for Conservation of Nature's Red List of Threatened Species = IUCN Red List (<http://www.redlist.org>). Additional information was gathered on the factors that threaten the populations in the survey areas.

Results

In this study, a total of 27 amphibian and reptile species belonging to 13 families were observed in Honaz Mountain National Park (Fig. 2). The species of amphibians and reptiles found in the research area, and their associated habitat types, are given in Tables 1 and 2. According to the Bern Convention, 12 of the amphibian and reptile species are listed in Appendix 2, and 15 species are on the Annex 3 list. According to the IUCN Red List criteria, one species is listed as VU (Vulnerable), 21 as LC (Least Concern), and one as DD (Data Deficient) [Fig. 3A]. Four species have not been evaluated yet (NE),

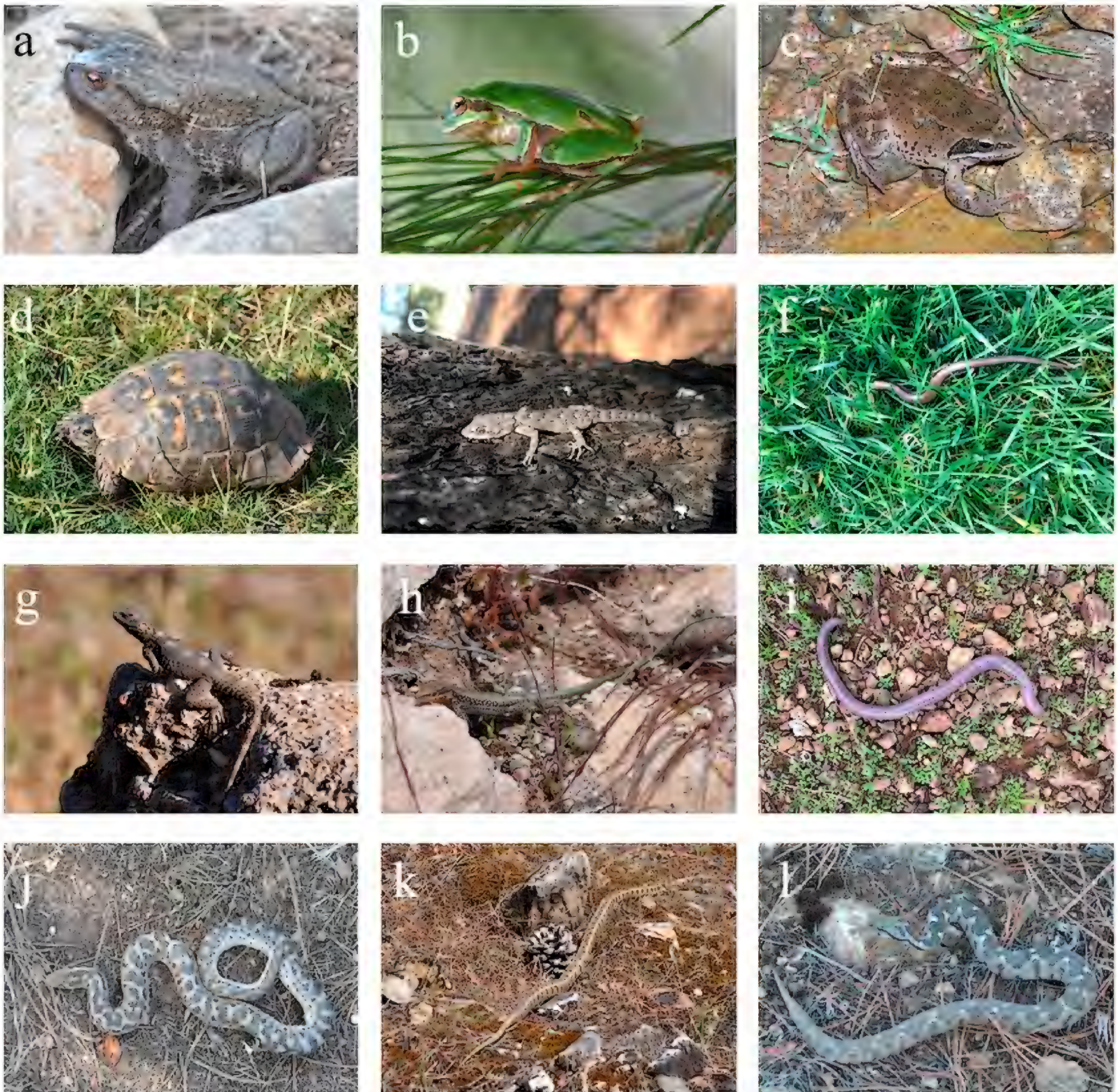


Fig. 2. Some of the amphibian and reptilian species in the study area: **(a)** *Bufo bufo*, **(b)** *Hyla orientalis*, **(c)** *Rana macrocnemis*, **(d)** *Testudo graeca*, **(e)** *Mediodactylus kotschyi*, **(f)** *Ablepharus kitaibellii*, **(g)** *Stellagama stellio*, **(h)** *Anatololacerta danfordi*, **(i)** *Blanus strauchi*, **(j)** *Eryx jaculus*, **(k)** *Natrix natrix*, and **(l)** *Montivipera xanthina*.

so they are not listed. In addition, according to CITES, two species are included in the Appendix 2 list and 25 are excluded from the list. The chorotype distributions of the species indicated that the amphibian and reptile species of Honaz Mountain National Park mostly belonged to the Turanian-Mediterranean and Eastern Mediterranean chorotypes (Vigna Taglianti et al. 1999; Sindaco et al. 2000) [Fig. 3B]. This study will inform the conservation of amphibian and reptile species in this area and contributes to the broader knowledge of Turkey's herpetofauna.

Discussion

Turkey has a rich flora and fauna due to its geological, topographical, and climatic characteristics. Herpetological

studies conducted for more than a century show that there is not enough information available on the herpetofauna of Turkey. On the one hand, in the last 20 years, new species and subspecies have been uncovered in Turkey and new localities have been recorded for the existing species (Ayaz et al. 2011; Göçmen et al. 2013; Avcı et al. 2015; Üzümlü et al. 2015; Bülbül et al. 2016). On the other hand, herpetology research has been limited to certain areas in Turkey despite the high number of divergent areas with differences in geographic isolation elements, and different ecosystems and ecological conditions. As a result, in the last 20 years, studies determining the amphibian and reptile species and their ecological preferences in the previously understudied regions have accelerated (Özdemir and Baran 2002; Afşar and Tok 2012; Özcan and Üzümlü 2013; Cihan and Tok 2014; Ege

Table 1. Amphibian species found in Honaz Mountain National Park and their characteristics.

Family	Species	Habitat	IUCN	Population status	Chorotype	BERN	CITES	Localities
Bufonidae	<i>Bufo bufo</i> (Linnaeus, 1758)	Forestlands, waysides, waterfronts, rocky terrains, meadowlands, croplands, gardens	LC	Stable	European	III	-	3, 15
Bufonidae	<i>Bufo variabilis</i> (Pallas, 1769)	Forestlands, waterfronts, meadowlands, croplands, gardens	DD	Undetermined/ unknown	Turano-European- Mediterranean	II	-	1, 2, 8, 10, 17, 18, 19
Ranidae	<i>Pelophylax bedriagae</i> (Camerano, 1882)	Forestlands, waysides, waterfronts, croplands	LC	Declining	Turano-European- Mediterranean	III	-	2, 6, 12, 20
Ranidae	<i>Rana macrocnemis</i> (Boulenger, 1885)	Waterfronts, croplands	LC	Declining	SW-Asiatic	III	-	9
Hylidae	<i>Hyla orientalis</i> (Bedriaga, 1890)	Forestlands, maquis shrublands, shrubbery	LC	Declining	European	II	-	2, 7

et al. 2015; Eksilmez et al. 2017; Kumlutas et al. 2017; Sarıkaya et al. 2017).

Most studies on the herpetofauna of Denizli province were carried out in the individual districts. For example, 17 reptile and amphibian species were found in the Babadağ district (Urhan et al. 1999); 24 in Acıpayam-Denizli (Urhan et al. 2003); 26 in Hambat (Urhan et al. 2004); and 22 around the Çal, Bekilli, and Baklan Districts (Urhan et al. 2006). Ünal et al. (2012) identified 25 amphibian and reptile species belonging to 15 families in Kale district. In this study, five anurans, two turtles, nine lizards, and 11 snakes (for a total of five amphibian and 22 reptile species) were observed in Honaz Mountain National Park. There are no current taxonomic controversies among these species. A previous study conducted about 20 years ago reported 21 amphibian and reptile species from Honaz Mountain National Park (Katılmış et al. 2002). In this study, *Hyla orientalis*, *Rana macrocnemis*, *Mauremys rivulata*, *Heremites auratus*, *Pseudopus apodus*, and *Telescopus fallax* were recorded for the first time in Honaz Mountain National Park. In addition, seven amphibian and 26 reptile species have been identified in Denizli province during 2002–2009 (Urhan et al. 2009). In this respect, the Honaz Mountain National Park includes 71.4% of the amphibian diversity and 80.8% of the reptile diversity in Denizli province.

Of the ~18,000 amphibian and reptile species distributed around the world, about 35% are on the IUCN Red List. According to IUCN criteria, among the total of 27 amphibian and reptile species found in the study area: one (4.76%) is Vulnerable (VU), 21 (66.7%) are Least Concern (LC), one (4.76%) is Data Deficient (DD), and 4 (28.57%) are not evaluated yet (IUCN 2018). According to the IUCN Red List, the populations of 10 of these

species are stable (37.04%), while the populations of four species are decreasing (14.81%), and there is no information on the population status of the remaining 13 species (48.15%). Therefore, it is necessary to determine the roles of various threats to the ecosystem and the current population status of the species of amphibians and reptiles that were found.

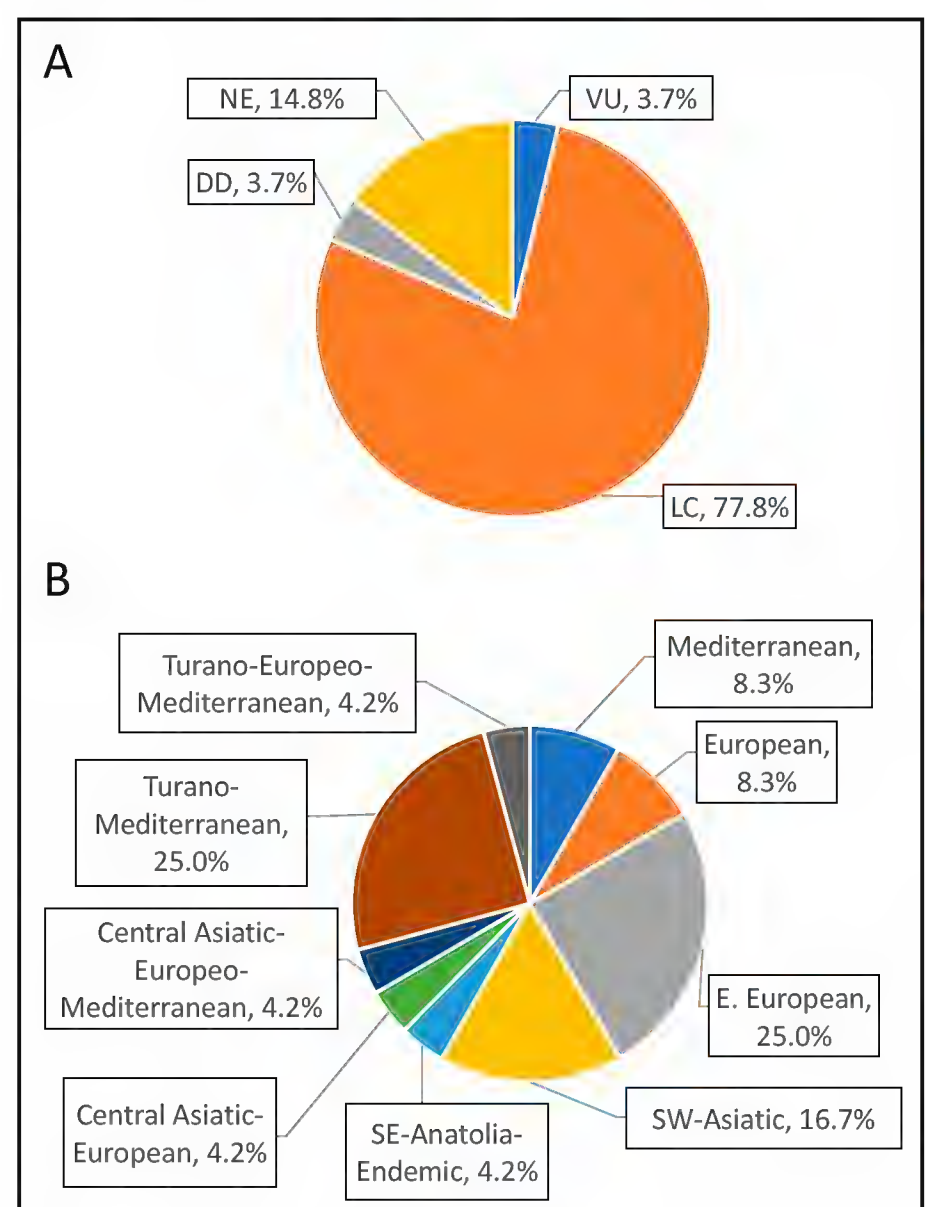

Fig. 3. Distribution of species according to IUCN categories (A) and their chorotypes (B).

Table 2. Reptile species found in Honaz Mountain National Park and their characteristics.

Family	Species	Habitat	IUCN	Population status	Chorotype	BERN	CITES	Localities
Testudinidae	<i>Testudo graeca</i> (Linnaeus, 1758)	Forestland, waterfronts, rocky terrain, meadowland, maquis shrubland, cropland, gardens, moorland	VU	Stable	Turano-Mediterranean	II	II	1, 3, 6, 8, 15, 16, 17, 19
Anguidae	<i>Pseudopus apodus</i> (Pallas, 1775)	Forestland, maquis shrubland, cropland, gardens	LC	Undetermined/Unknown	Turano-Mediterranean	II	-	4, 6, 10, 16
Geoemydidae	<i>Mauremys rivulata</i> (Valenciennes in Bory de Saint-Vincent, 1833)	Waterfronts, creeks	LC ₁	Undetermined/Unknown	Turano-Mediterranean	III	-	2
Agamidae	<i>Stellagama stellio</i> (Linnaeus, 1758)	Wayside, rocky terrain	LC	Stable	E. Mediterranean	II	-	3, 5, 18, 21
Gekkonidae	<i>Mediodactylus kotschyi</i> (Steindachner, 1870)	Forestlands, waysides, open slopes, valley slopes, rocky terrains, maquis shrublands, croplands, gardens, residential areas	LC	Undetermined/Unknown	E. Mediterranean	II	-	1, 8, 10, 17, 19
Lacertidae	<i>Anatololacerta danfordi</i> (Günther, 1876)	Forestlands, waterfronts, open slopes, valley slopes, rocky terrains, meadowlands, maquis shrublands, croplands	LC	Stable	SE-Anatolia-Endemic	III	-	2, 12
Lacertidae	<i>Lacerta trilineata</i> (Bedriaga, 1886)	Forestlands, rocky terrains, croplands, bushlands, moorlands, semi-desert areas	LC	Stable	E. Mediterranean	II	-	2, 7, 12
Lacertidae	<i>Ophisops elegans</i> (Ménétries, 1832)	Forestland, rocky terrains, croplands, bushlands, moorlands, semi-desert areas	-	Undetermined/Unknown	E. Mediterranean	II	-	1, 3, 9, 10, 14, 18, 22
Scincidae	<i>Ablepharus kitaibelii</i> (Bibron and Bory St-Vincent, 1833)	Forestlands, rocky terrains, meadowlands	LC	Stable	E. Mediterranean	II	-	4, 5, 13
Scincidae	<i>Heremites auratus</i> (Linnaeus, 1758)	Rocky terrains	LC	Stable	SW-Asiatic	III	-	1, 11
Amphisbaenidae	<i>Blanus strauchi</i> (Bedriaga, 1884)	Forestlands, rocky terrains, croplands	LC	Undetermined/Unknown	Mediterranean	III	-	3, 11, 17, 21
Typhlopidae	<i>Xerotyphlops vermicularis</i> (Merrem, 1820)	Forestlands, waterfronts, rocky terrains, meadowlands	LC	Undetermined/Unknown	Turano-Mediterranean	III	-	4, 11, 17,
Colubridae	<i>Dolichophis jugularis</i> (Linnaeus, 1758)	Waterfronts, open slopes, valley slopes, rocky terrains, cropland, gardens	LC	Stable	SW-Asiatic	II	-	2, 12
Colubridae	<i>Dolichophis caspius</i> (Gmelin, 1789)	Open slopes, rocky terrains, meadowlands, croplands, gardens	LC	Undetermined/Unknown	Turano-Mediterranean	III	-	3, 17, 21
Colubridae	<i>Hemorrhois nummifer</i> (Reuss, 1834)	Open slopes, rocky terrains	LC	Undetermined/Unknown	Turano-Mediterranean	III	-	12
Colubridae	<i>Eirenis modestus</i> (Martin, 1838)	Rocky terrains	LC	Stable	SW-Asiatic	III	-	3, 21

Table 2 (continued). Reptile species found in Honaz Mountain National Park and their characteristics.

Family	Species	Habitat	IUCN	Population status	Chorotype	BERN	CITES	Localities
Colubridae	<i>Telescopus fallax</i> (Fleischmann, 1831)	Forestlands, bushlands, rocky terrains	LC	Undetermined/Unknown	Turano-Mediterranean	III	-	5, 8
Colubridae	<i>Elaphe sauro-mates</i> (Pallas, 1811)	Forestlands, waysides, open slopes, rocky terrains, meadowlands, croplands, gardens, bushlands, moorlands	LC	Undetermined/Unknown	Turano-European-Mediterranean	III	-	3
Colubridae	<i>Natrix natrix</i> (Linnaeus, 1758)	Forestlands, waterfronts, meadowlands	LC	Undetermined/Unknown	Central Asiatic-European-Mediterranean	III	-	2
Colubridae	<i>Natrix tessellata</i> (Laurenti, 1768)	Waterfronts	LC	Declining	Central Asiatic-European	II	-	12
Boidae	<i>Eryx jaculus</i> (Linnaeus, 1758)	Forestlands, open slopes, valley slopes, rocky terrains	LC	Undetermined/Unknown	Mediterranean	III	II	13
Viperidae	<i>Montivipera xanthina</i> (Gray, 1849)	Open slopes, valley slopes, rocky terrains	LC	Stable	E. Mediterranean	II	-	4

As indicated by field studies, all of the threats to amphibian and reptile diversity are found to originate from anthropogenic degradation of the environment (Curic et al. 2018; Hosseinzadeh et al. 2018). Destruction of habitat is the major threat for many species. Considering that the terrestrial frog and reptile species are mainly distributed in mountain slopes, as well as rocky and stony areas, mining activities pose a significant threat to biodiversity. Destruction and restriction of the habitats of amphibian and reptile species may cause them to become trapped in certain areas over the long term. The competition of species having a common niche will cause stress in terms of food resources and reproduction, and species that are trapped in certain areas will be more sensitive to changes in the ecosystem. Two other problems are the agricultural activities and domestic pollution which affect the species that are dependent on aquatic habitats. The species living in irrigation channels in agricultural lands are directly exposed to pesticides. During our field studies, packages of agricultural pesticides were found in many habitats, such as irrigation channels and streams. Another important threat for the reptiles is that local people do not recognize the non-venomous reptile species and kill reptiles indiscriminately due to mis-information. Most snake species encountered in the field or in different environments are killed despite being harmless. Even the European Glass Lizard (*Pseudopodus apodus*) is known by the local people as the “iron snake” and it is often killed due to the belief that it is venomous. As reptiles do not have mimic muscles and they have scaly structures, people often consider them to be “cold-faced animals.” Another problem observed in the survey area is that amphibians and reptiles are often crushed by road traffic.

Several recommendations for the conservation of species are given here.

- Information and training activities should be initiated in the habitat areas which are intensively used by people, such as recreational areas. In addition, Natural Life Protection Schools, supported by various institutions and associations, should be expanded and brought to a level that will appeal to more people.
- Modification or destruction of habitats should be prevented. If a study on habitat is conducted, at a minimum it should be carried out under the supervision of a commission of biologists and should be done outside of the breeding season.
- Most specimen mortalities occur during the migration to wetland ecosystems during the reproductive season. During migration, many adult individuals are destroyed on the roads due to heavy traffic. For this reason, barriers should be built around the main arteries in the wetland ecosystems and their channels; and similar structures should be built to ensure that individuals migrating to the wetland can pass from safe areas to the wetlands.
- Predator species not found in the natural environment should not be released to the wetland ecosystems. Numerous studies have shown that species added to the environment for biological control are harmful to the ecosystem over time, and often a struggle ensues to remove these species from the environment. For this reason, steps should be taken to ensure that these predators are removed as soon as possible.
- Due to the agricultural activities around the wetlands, many chemical pollutants are introduced into the water bodies. Therefore, when controlling harmful plants and insects, only herbicides and insecticides approved by the Republic of Turkey Ministry of Agriculture and Forestry should be used, and disposal of

their waste materials should be in an isolated environment away from the water bodies.

- The environment is continuously being loaded with chemical substances released by anthropogenic activities that may directly or indirectly affect herpetofaunal populations. The geno- and ecotoxicological effects of these substances on living things should be further investigated.

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Müge Gidiş is an Assistant Professor in the Department of Biochemistry at the University of Dumlupınar (Kütahya, Turkey). Müge is interested in the molecular ecology and conservation biology of reptiles and amphibians. Her recent research projects focus on the comparative phylogeography of amphibians and reptiles.



Eyup Başkale is a Professor in the Department of Biology at the University of Pamukkale (Denizli, Turkey). His current research seeks to understand animal ecology and distribution, with special focus on the conservation of amphibians and reptiles living in Turkey. Eyup currently coordinates several research projects focusing on threatened species in Turkey.

Appendix 1. Coordinates and altitudes of the study locations at Honaz Mountain National Park, Turkey.

Locality no.	Longitude	Latitude	Altitude (m)
1	29°15.646'E	37°47.216'N	376
2	29°19.080'E	37°44.227'N	1,101
3	29°15.999'E	37°43.358'N	1,345
4	29°14.361'E	37°41.585'N	1,156
5	29°17.545'E	37°42.625'N	1,427
6	29°14.031'E	37°44.044'N	1,138
7	29°14.692'E	37°39.455'N	1,200
8	29°18.739'E	37°42.911'N	1,645
9	29°18.828'E	37°43.486'N	1,622
10	29°17.338'E	37°42.487'N	1,402
11	29°16.884'E	37°42.254'N	1,405
12	29°17.556'E	37°44.199'N	785
13	29°15.847'E	37°41.047'N	1,909
14	29°15.865'E	37°39.939'N	1,592
15	29°13.089'E	37°44.586'N	902
16	29°18.984'E	37°41.988'N	1,742
17	29°17.642'E	37°40.152'N	2,025
18	29°13.609'E	37°42.896'N	1,070
19	29°16.014'E	37°47.440'N	362
20	29°20.141'E	37°43.829'N	1,109
21	29°16.090'E	37°44.489'N	808
22	29°14.095'E	37°38.874'N	1,272



Patterns of growth and natural mortality in *Lysapsus bolivianus* (Anura, Hylidae, Pseudae) in an environmental protection area in the estuary of the Amazon River

^{1,*}Júlio C. Sá-Oliveira, ²Carlos E. Costa-Campos, ³Andréa S. Araújo, and ⁴Stephen F. Ferrari

¹Research Nucleus in Fisheries and Aquaculture-NEPA, Laboratory of Limnology and Ichthyology, Federal University of Amapá (UNIFAP), Campus Universitário Marco Zero do Equador, Rod. Juscelino Kubitschek, Km 02, CEP 68903-419 Macapá, Amapá, BRAZIL ²Herpetology Laboratory, Federal University of Amapá (UNIFAP), Campus Universitário Marco Zero do Equador, Rod. Juscelino Kubitschek, Km 02, CEP 68903-419 Macapá, Amapá, BRAZIL ³Zoology Laboratory, Federal University of Amapá (UNIFAP), Campus Universitário Marco Zero do Equador, Rod. Juscelino Kubitschek, Km 02, CEP 68903-419 Macapá, Amapá, BRAZIL ⁴Department of Ecology, Federal University of Sergipe – UFS, São Cristóvão, BRAZIL

Abstract.—Recent reviews indicate that about one-third of amphibian species are threatened with extinction. Many of these species inhabit tropical areas in developing countries where deforestation and the degradation of natural bodies of water are major threats. *Lysapsus bolivianus* is a poorly known amphibian found throughout much of the central Amazon basin between Bolivia and the Amazon estuary, where it is subject to extensive anthropogenic pressures. The present study was based on samples of this species in an environmental protection area. The data obtained is important for understanding the population structure with respect to size, growth parameters (K , $A_{0.95}$, L_{∞} , $\emptyset'$, SVL_{max}), and natural mortality of the species. The results showed a sexual dimorphism in size, with females being larger. Both sexes presented fast growth rates ($K_{Male} = 0.71 \text{ year}^{-1}$; $K_{Female} = 0.70 \text{ year}^{-1}$), reaching asymptotic sizes ($SVL_{\infty Male} = 21.20 \text{ mm}$; $SVL_{\infty Female} = 25.60 \text{ mm}$) in less than twelve months, and longevity of <5 years. The species completes its metamorphosis in 20 days, reaching adult age at one month. The estimated natural mortality was 0.64 year^{-1} for males and 0.65 year^{-1} for females. The precocity of this species, as well as the frequency of individuals of various ages and sizes during the whole year, suggests it has developed tactics that allow its survival in this environment with small sizes (average 1.7 cm), which characterizes it as an r-strategist. Anthropogenic pressures in areas where *L. bolivianus* lives in Brazil, as in the area of the present study, make the species vulnerable because they increase its exposure to predators, reduce its breeding sites, and increase its mortality from agricultural pesticides.

Keywords. Amapá, Amphibia, Brazil, Eastern Amazon, population structure

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Introduction

While amphibian research has advanced considerably in recent decades throughout the world, the ecology of most species is still only poorly understood. This is reflected, for example, in the large number of amphibian species classified as Data Deficient by the IUCN (2016). The comprehensive Global Amphibian Assessment (GAA) estimated that more than 30% of the 7,881 recognized amphibian species are currently threatened with extinction, and that several hundred may already be extinct (Barnovsky et al. 2011; IUCN 2016).

The population dynamics of amphibians is still poorly known in comparison with the diversity of this group

(Measey et al. 2016) or other poikilothermic vertebrates, such as fishes and reptiles (Kozłowski and Teriokhin 1999; Shine 2010; Camargo et al. 2010; Loyola et al. 2008). This lack of data is a major obstacle to the development of effective conservation measures (Stuart et al. 2004).

The life history strategies of a species are reflected in a characteristic set of biological and demographic traits, such as age at first sexual maturation, fertility and mortality rates, reproductive patterns, and social organization (Stearns 1992; Ricklefs 1977). Growth and mortality rates may be especially important for the understanding of population structure and dynamics, and the capacity of a species to cope with environmental disturbance

Correspondence. *juliosa@unifap.br

(Radtke and Hourigan 1990; Pauly 1998).

In most tropical organisms, the assessment of growth in rigid structures, such as bones, scales, and woody stems, is impeded by the relative stability of the climate and associated ecological variables (Boujard et al. 1991; Marangoni et al. 2009). In this case, growth parameters may be estimated indirectly through data on parameters such as body size, which are more easily obtained from wild populations. These data can provide insights into the typical body sizes of different age groups, and the definition of cohorts (Basson et al. 1988).

A number of non-linear models have been proposed to describe growth patterns in animals, such as the Brody, von Bertalanffy, and Gompertz functions, and logistic procedures. The Bertalanffy model is the most popular model for analyzing animal population dynamics. It is based on the assumption that growth can be estimated from the difference between the anabolic and catabolic rates of an animal (Bertalanffy 1957; Hota 1994).

The frog *Lysapsus bolivianus* Gallardo, 1961 is a semiaquatic anuran with both nocturnal and diurnal habits, which inhabits the water surface in patches of floating aquatic vegetation (Bosch et al. 1996; Garda et al. 2010; Santana et al. 2013). This species is small in size (mean SVL = 17.6 mm), and is widely distributed in the Amazon basin, ranging from the mouth of the Amazon River in Brazil to northern Bolivia (Frost 2018). It is found in the Rio Curiaú Environmental Protection Area (Rio Curiaú EPA) in Amapá state, northern Brazil, the location of the present study (Melo-Furtado et al. 2014). This protected area has been extensively impacted by human activities, such as deforestation, unregulated fishing, construction of buildings, the accumulation of domestic refuse, landfill of floodplains, and the indiscriminate use of agricultural pesticides in the surrounding areas. All these processes may impact the local biota, especially the anurans, such as *L. bolivianus*.

The present study evaluated the growth and mortality parameters of the *L. bolivianus* population of the Rio Curiaú EPA, together with estimates of longevity and growth performance. These data will hopefully contribute to the development of effective conservation strategies for the study species and other amphibians, as well as the study area in general.

Materials and Methods

Study Area

The present study was conducted in the Rio Curiaú Environmental Protection Area (00°09'00.7"N, 51°02'18.5"W), or Rio Curiaú EPA, which lies to the north of the Amazon River estuary in the state of Amapá, northern Brazil. The Rio Curiaú EPA encompasses 21,700 ha, an area dominated by aquatic systems, such as rivers and seasonal lakes. The local vegetation is mainly Cerrado savanna and floodplain forest. The region's

climate is humid equatorial (*Am*) in the Köppen-Geiger classification system, with a mean monthly temperature of 27.6 °C (range: 25.8–29 °C) and mean annual rainfall of approximately 2,850 mm, with a monsoon period between February and May when the monthly precipitation is around 400 mm (Alvares et al. 2013). The number and sizes of the ponds found within the study area decrease considerably during the dry season.

Sample Collection

Between January and December 2015, frogs were captured randomly by hand during the night, using 9 V flashlights. Frogs were collected by active searches along five 1 km transects in floating vegetation (*Nymphoides indica* [L.] Kuntze and *Salvinia auriculata* Aubl.). The transects were separated by a distance of at least 50 m and were walked by three researchers during each survey (Crump and Scott 1994). The frogs captured were examined to determine their sex and age (adult or juvenile), based on the presence of the nuptial sac in males and the distended or flaccid abdomen (before or after spawning) of the mature females, and their positions in amplexus.

The snout-vent length (SVL, in mm) of each specimen was measured using a tape measure and calipers, and the weight (*Wt*, in g) was recorded using a spring balance (0.01 g precision). Sample collection was authorized by the Brazilian Environment Institute (IBAMA) and the Information and Authorization System (SISBIO) of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through license number 34238-1. After measurements were taken, all individuals were released at the capture site.

Statistical Analyses

Deviations in the sex ratio were evaluated using a Chi-square test with Yeats' correction. The SVL values were grouped into classes to permit the visualization of the differences between adults and juveniles, and between mature males and females. The difference in the mean body size (SVL) between males and females was analyzed using a *t* test. For this, the assumptions of normality and homoscedasticity were tested *a priori* using the Kolmogorov-Smirnov and Bartlett tests, respectively. A significance level of 5% was considered in all cases.

The total length-weight relationship was determined by the Sparre et al. (1989) allometric equation $Wt = a * L^b$, where *Wt* = body weight (g), *L* = SVL (mm), and 'a' and 'b' = regression constants. Growth parameters were based on the Von Bertalanffy equation, $SVL = SVL_{\infty} \times (1 - e^{-k(t-t_0)})$ [Sparre and Venema 1998], where *SVL* = total snout-vent length (mm) at age *t*, *SVL*_∞ = asymptotic snout-vent length (mm), *K* = growth rate (year⁻¹), *t* = the age in years, and *t*₀ = the nominal age at metamorphosis, assumed to be zero. The constants *K* and *SVL*_∞ were estimated by the Ford-Walford model (Ford 1933; Walford 1946).

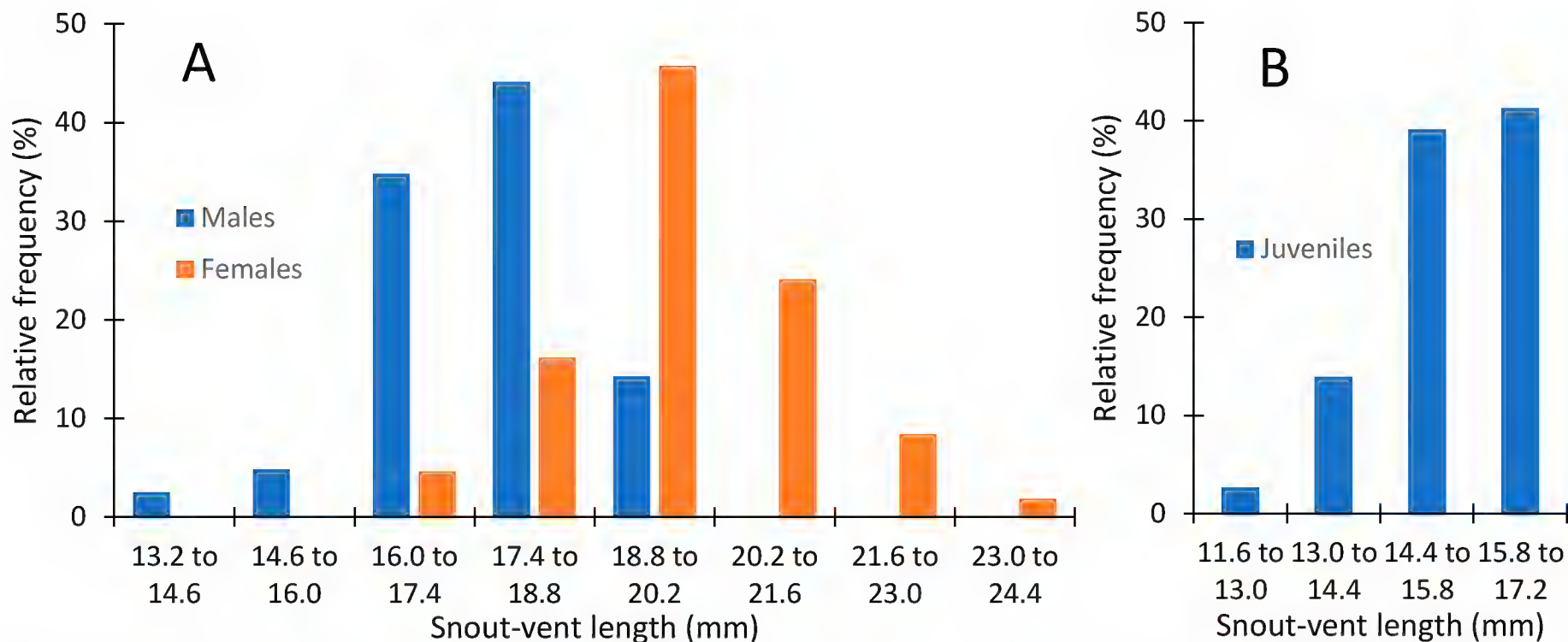


Fig. 1. Relative frequency of the body size classes (SVL, snout–vent length; mm) recorded in the (A) adult males and females and (B) juveniles of the *Lysapsus bolivianus* population from the Rio Curiaú EPA on the estuary of the Amazon River, in northern Brazil.

Longevity ($A_{0.95}$ or t_{max}) was calculated using the Taylor (1958) equation, $A_{0.95} = t_0 + (2.996/K)$, and natural mortality (M) was estimated using the Hoenig (1983) equation which is based on the empirical relationship observed between M and the maximum age described by the equation $LnM = 1.46 - 1.01[Ln(t_{max})]$, where t_{max} = the maximum age in the population, and M = the natural rate of mortality. The asymptotic weight (W_{∞}) was estimated by converting L_{∞} to the corresponding weight using the Pauly (1998) formula for the length-weight relationship ($W_{\infty} = a * SVL_{\infty}^b$). The growth performance (ϕ') was estimated by the Pauly and Munro (1984) formula: $\phi' = \log k + 2 \log SVL_{\infty}$. Juveniles were analyzed separately from the adults due to their much faster juvenile growth rates (mean = 16.0 ± 3.6 days). As the sex of the juveniles could not be determined, the data were pooled for this age class.

Results

A total of 308 mature *L. bolivianus* individuals were examined, together with 71 individuals classified as juveniles. Overall, mature males ($n = 188$) were significantly more abundant than mature females, with $n = 120$ (X^2 [with Yates' correction] = 14.57; $df = 1$; $p = 0.001$). However, the females were significantly larger, on average, than males, with a mean SVL in the females of 19.81 ± 1.35 mm (range = 16.40–23.48 mm) versus $17.60 \pm$

1.13 mm (14.11–20.17 mm) in the males ($t = 15.26$; $df = 306.0$; $p < 0.0001$). Clear peaks in body size were observed in both sexes (Fig. 2), with 79.0% of the adult males having an SVL of 16.0–18.8 mm, and 78.2% of the females at 18.8–21.6 mm. The juveniles presented a mean SVL of 15.42 ± 1.07 mm (range: 12.00–16.96 mm), with 81.13% of the specimens lying between 14.4 and 17.2 mm (Fig. 1).

Highly significant coefficients of determination were recorded for the total length-weight relationships in both adult males ($R^2 = 0.63$; $F_{(2,185)} = 67.29$; $p < 0.0001$) and females ($R^2 = 0.74$; $F_{(2,117)} = 521.51$; $p < 0.0001$), indicating different models for the two sexes. The b values of males and females were both lower than 3, which indicate negative allometric growth (Fig. 2).

The relationship between the mean SVL at age t and $t + 1$ ($SVL + 1$) was described adequately by Walford's equation: $SVL_{t+1} = 12.971 + 0.4192 SVL$, $R^2 = 0.989$ for males and $SVL_{t+1} = 12.149 + 0.5198 SVL$, $R^2 = 0.959$ for females (Fig. 4). The intersection between the function and the diagonal drawn through the origin provides the value of L_{∞} , which was 21.20 mm in males and 25.47 mm in females. Based on the formula $L_{\infty} \sim L_{max} / 0.95$ (Taylor 1958), the estimated values of SVL_{∞} were 21.17 mm for males, and 24.65 mm for females, values which are very close to those derived from the graphs (Fig. 3). Estimated growth parameters are presented in Table 1. In general, the parameters were similar between males and females,

Table 1. Growth parameters for *Lysapsus bolivianus* specimens from the Rio Curiaú EPA in Amapá, Brazil. SVL_{max} = maximum length; SVL_{∞} = asymptotic length; k = growth constant; ϕ' = growth performance; M = mortality; $A_{0.95}$ = longevity; n = sample size.

Parameter	Males (n = 188)	Females (n = 120)	Juveniles (n = 71)
K (year ⁻¹)	0.71	0.70	0.81
SVL _∞ (mm)	21.20	25.60	59.50
ϕ'	2.50	2.65	3.45
A _{0.95}	4.20	4.28	3.70
SVL _{max} (mm)	20.17	23.48	16.96
M (year ⁻¹)	0.64	0.65	12.86

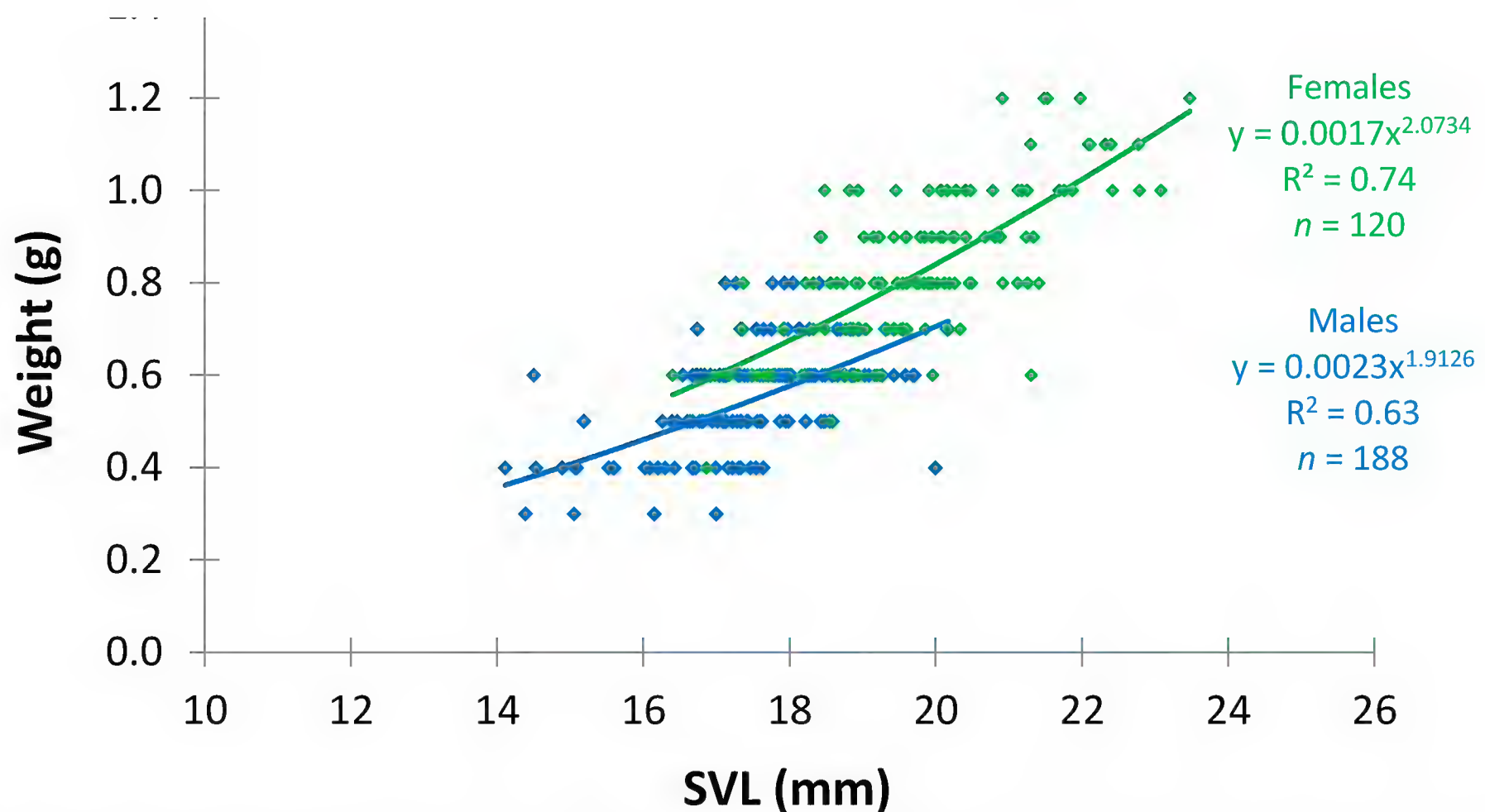


Fig. 2. Weight-length relationships in adult male and female *Lysapsus bolivianus* from the Rio Curiaú EPA on the estuary of the Amazon River, in northern Brazil.

although asymptotic size and growth performance varied between the sexes. Growth rates, longevity, and natural mortality were equal in the sexes.

Males grew 2.22 mm, on average, from an age of 3 to 6 months, 0.26 mm from 6 to 9 months, and only 0.04 mm from 9 to 12 months (Table 2). In females, growth over these same intervals was 2.80 mm, 0.30 mm, and 0.10 mm, respectively. This variation was shown in the Bertalanffy growth curves (Fig. 4), which followed distinct patterns in the males and females.

Discussion

The predominance of males recorded in the present study was consistent with the findings of Melo-Furtado et al. (2014). The male-biased sex ratio in *L. bolivianus* may be advantageous for the fertilization of the largest possible number of eggs. Other studies have related deviations in sex ratio to factors including differential growth and mortality, as well as fluctuations in the availability of nutrients and behavioral variations, all of which may have varying influences on the proportion of the sexes

at different stages of development (Hamilton and Zuk 1982; Vazzoler 1996; Kraab and Pen 2002; Fawcett et al. 2011; Booksmythe et al. 2013).

The growth rates of both male and female *L. bolivianus* were relatively high (> 0.5), which is typical of species found in highly seasonal habitats, such as that of the study area, as well as those that suffer high rates of predation (Lowe-McConnell 1999). These species grow rapidly, reaching maturity sooner with smaller asymptotic body lengths than larger species with slower growth rates (Pauly 1998).

A reduced asymptotic length, while determined genetically, may also be influenced by variables such as the food supply and population density (Parker 1983; Hubbell and Johnson 1987). Pauly (1998) concluded that low values are typical of tropical species and may be attributed to the combination of a number of factors, particularly temperature, given that higher temperatures accelerate growth and metabolic rates but tend to decrease the asymptotic size (Lomolino and Perault 2007). Faster growth to maturity may also be a strategy to compensate for predation pressure (Reznick et al. 1996).

The natural mortality rates in both sexes were moderate to high, and varied proportionately with growth rates, indicating that the principal causes of mortality in this species are predation and longevity, related to its rapid life cycle (Keiber 1932; Pauly 1998). While the *L. bolivianus* males grow faster than the females, both sexes have similar longevity, which favors reproductive success in a highly seasonal environment that is characterized by long periods of drought.

Table 2. Average snout-vent length at different ages calculated for adult male and female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil.

	Average SLV (mm) at age indicated			
	3 months	6 months	9 months	12 months
Male	18.68	20.90	21.16	21.20
Female	22.40	25.20	25.50	25.60

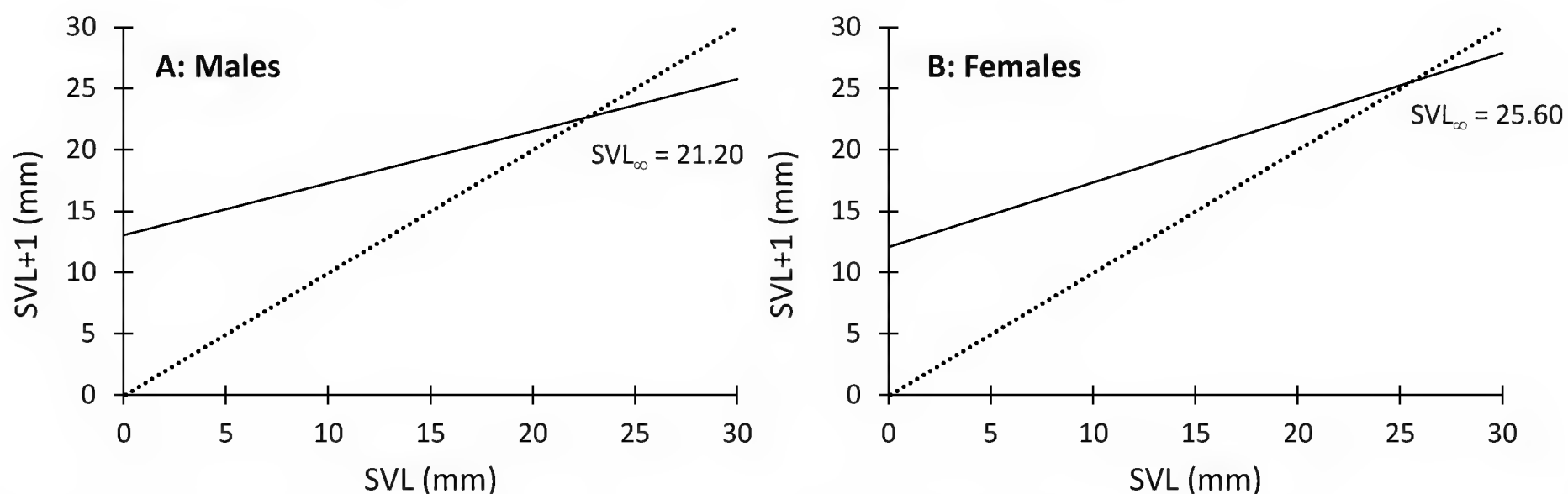


Fig. 3. Plot of the Ford-Walford estimates of growth parameters (SVL_{∞} , k) of adult (A) male and (B) female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil. The values were estimated by the linear regressions between SVL and SVL+1 for each gender, as $SVL_{\infty} = (a/1-b)$ and $K = -\log_e b$.

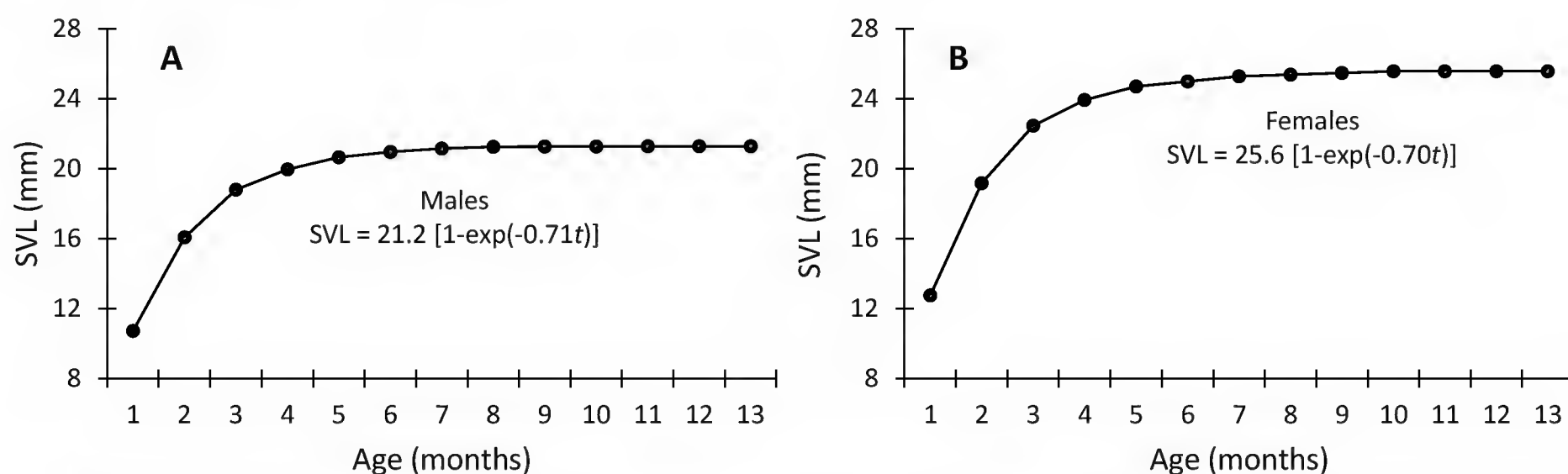


Fig. 4. Von Bertalanffy's growth curves for (A) male and (B) female *Lysapsus bolivianus* from the Rio Curiaú EPA in Amapá, Brazil.

Conclusions

All parameters analyzed indicate an *r* type of life history strategy in *L. bolivianus*. This is typical of species that inhabit highly unstable environments, such as that of the present study area, which is subject to marked seasonal fluctuations in water levels, reinforcing the rapid growth, small size, and reduced longevity of these frogs. Anthropogenic pressures in the areas occupied by *L. bolivianus* in Brazil, such as the present study area, augment the vulnerability of this species due to increasing exposure to predators, the reduction in available breeding sites, and increasing mortality through the use of agricultural pesticides.

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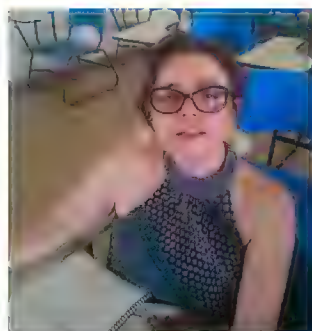
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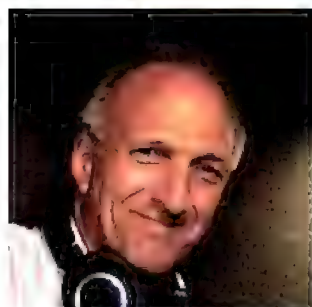
Júlio C. Sá-Oliveira is a biologist, with a Doctorate in Aquatic Ecology and Fisheries, and is currently a teacher at the Federal University of Amapá-Brazil, Júlio has experience in the area of the ecology of aquatic environments, with emphasis on bioecology, water quality assessment, and modeling of ecosystems, populations, and communities.



Carlos E. Costa-Campos has a Ph.D. in Psychobiology from the Federal University of Rio Grande do Norte, Brazil. Carlos has experience in the area of zoology (with an emphasis on amphibians and reptiles), and is active in research on the natural history, ecology, behavior, and conservation of herpetofauna. Currently he is a teacher at the Federal University of Amapá, Brazil.



Andrea S. Araújo has a Ph.D. in Psychobiology from the Federal University of Rio Grande do Norte, Brazil, and is an Adjunct Professor III of the Federal University of Amapá, also in Brazil. Andrea has experience in zoology, with an emphasis on vertebrate zoology, working mainly on the behavior, ethnozoology, and ecology of vertebrates.



Stephen F. Ferrari has a bachelor's degree (University of Durham, 1983) and Ph.D. (University of London, 1988) in Biological Anthropology. Stephen is currently an Associate Professor I at the Federal University of Sergipe, Brazil. He is also an ad-hoc consultant for CAPES and ICMBio, and a member of the IUCN Primate Specialist Group. Stephen has experience in ecology, with emphasis on primatology, working mainly in ecology, conservation, animal behavior, habitat fragmentation, and environmental education.



Persistence of snake carcasses on roads and its potential effect on estimating roadkills in a megadiverse country

Laura Ximena Cabrera-Casas, Lina Marcela Robayo-Palacio, and *Fernando Vargas-Salinas

Grupo de investigación en Evolución, Ecología y Conservación EECO, Programa de Biología, Facultad de Ciencias Básicas y Tecnologías, Universidad del Quindío, Armenia, COLOMBIA

Abstract.—The persistence of fauna carcasses on roads has been considered one of the most relevant factors influencing estimates of road mortality in different taxonomic groups. However, there is a lack of information in this regard in most Neotropical countries. The aim of the present research is to describe and quantify the persistence of snake carcasses on two Colombian roads and its potential relationships with animal body size and the frequency of vehicular traffic. Additionally, to illustrate the importance of correcting roadkill rate estimates for carcass persistence, the roadkill rate of snakes on a secondary road in the study area is recalculated using the results of carcass persistence time, instead of a previously selected arbitrary value (i.e., 7 days). To estimate the carcass persistence time, eighty-one snake carcasses of diverse body sizes (mean length = $46.90 \text{ cm} \pm (\text{SD}) 38.46$, range = 10.1–224 cm) were placed over sampling points distributed equally on two roads with different traffic frequencies (a primary road with > 2,500 vehicles/day and a secondary road with < 1,000 vehicles/day). Snake carcass degradation was monitored until their disappearance from the road surface. The median persistence time of carcasses on roads was 7.16 h (5.20 h and 14.16 h on the primary and secondary roads, respectively). According to field experiments, around 75% of the carcasses can disappear from the road surface within 30 h after a snake has been killed. The principal cause of the disappearance of carcasses during the day was degradation due to vehicular traffic. As the carcasses tended to increase in size, the difference in their persistence between types of roads increased, with lower persistence on the primary road than on the secondary road. The conclusion from these observations is that excluding carcass persistence time from snake roadkill rate calculations leads to high underestimates of mortality. Therefore, the problem of snakes that are roadkill on the road infrastructure in Colombia is much greater than previously considered.

Keywords. Andes, Colombia, mortality, Reptilia, road ecology, Serpentes, Squamata, vehicular traffic

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Introduction

Among the ecological effects attributed to the presence of roads is animal mortality due to collision with vehicles (Forman et al. 2003; van der Ree et al. 2015). Fauna roadkill rates have been estimated in thousands to millions of animals per year (Huijser and McGowen 2010; Marsh and Jaeger 2015). For example, in Australia, it has been estimated that up to 12 million vertebrates are killed yearly on roads, while in the United States the estimates are 1 million vertebrates per day (Bennett 1991; Forman and Alexander 1998). However, those roadkill rates can be severely underestimated (Loss et al. 2014; Ruiz-Capillas et al. 2015; Santos et al. 2016) given methodological biases and that many carcasses disappear before being registered by researchers due to factors such as rain, wind, scavenging activity, or vehicular traffic

(Bafaluy 2000; Ratton et al. 2014; Slater 2002; Taylor and Goldingay 2004). In fact, roadkill estimates are generally evaluated based upon time ranges of carcass persistence on roads that have been arbitrarily selected or with conservative approximations (Klöcker et al. 2006).

In Latin America, several studies have documented wildlife roadkill rates (e.g., Novelli et al. 1988; Pinowski 2005; Delgado 2007; Coelho et al. 2008; Barri 2010; Rojas-Chacón 2010; Vargas-Salinas et al. 2011; Contreras-Moreno et al. 2013; De La Ossa-Nadjar and De La Ossa 2013; Seijas et al. 2013). Nevertheless, few studies have tested the factors affecting carcass persistence on the roads and hence, have not quantified the discrepancy between the number of carcasses counted by researches and the real number of animals killed on the roads (Santos et al. 2011; Santos et al. 2016; Teixeira et al. 2013). This is concerning because the accurate

Correspondence. cabreracasaslx@gmail.com (LXC); lmrobayo.palacio@gmail.com (LMR); *fvargas@uniquindio.edu.co (FVS)

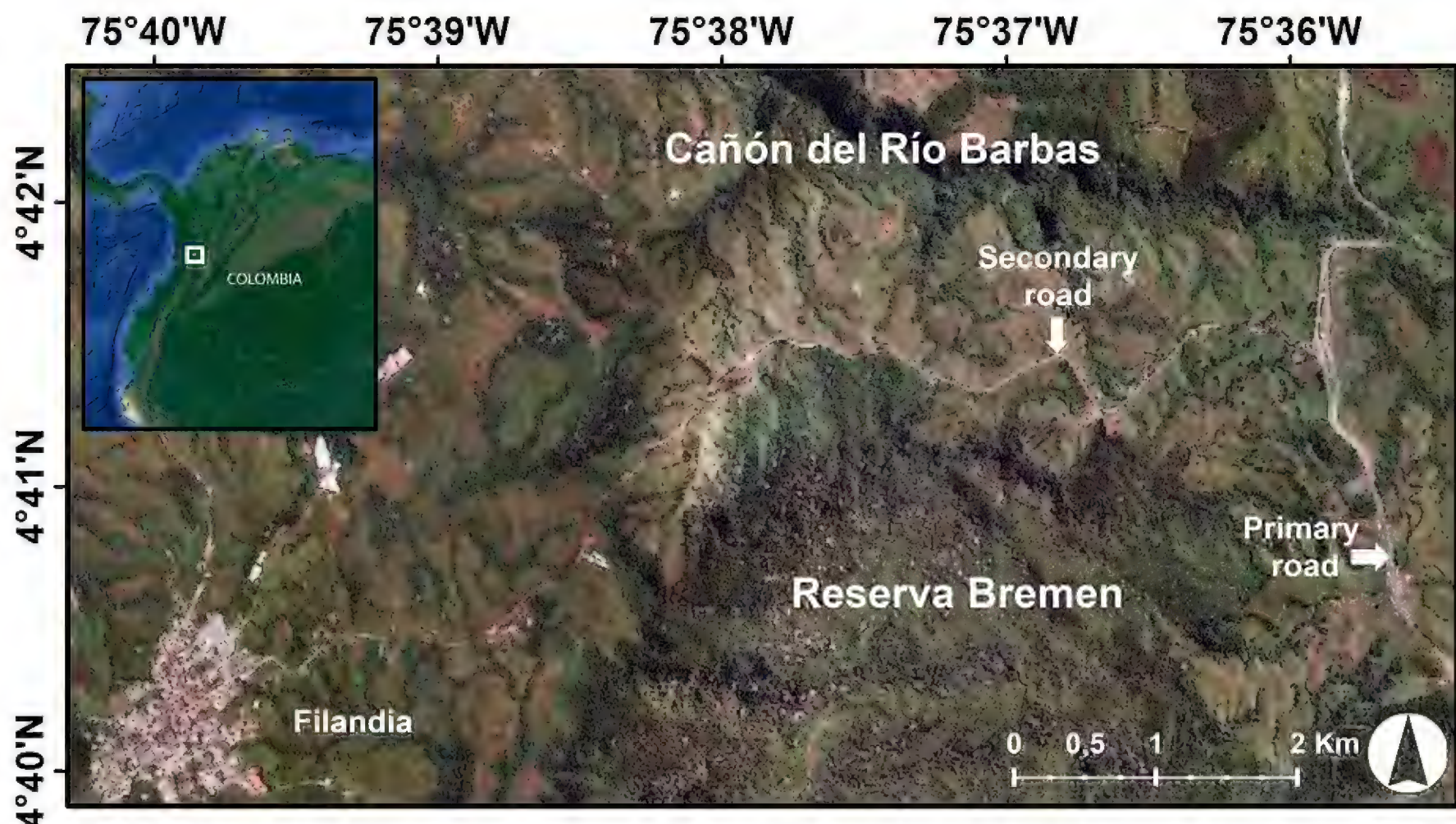


Fig. 1. Geographic location of the study area. Primary road (*Autopista del Café*) and the secondary road connecting *Autopista del Café* with the town of Filandia in the department of Quindío, Central Andes of Colombia. Adapted from SIG Quindío 2016. <http://190.85.164.56/sigquindioiii/>

quantification of roadkill rates is crucial for assessing the negative impact of roadkills in animal populations, and for optimizing the allocation of the limited economic resources for management and conservation (Slater 2002; Fahrig and Rytwinski 2009).

Snakes are one of the most commonly killed vertebrates on roads, which has been attributed to several non-exclusive factors (Andrews et al. 2008). For instance, snakes may actively seek out roads for thermoregulation (Rosen and Lowe 1994; Ashley and Robinson 1996) and they are especially vulnerable to being killed because they tend to freeze when facing an anthropogenic stimulus such as the artificial light of approaching vehicles (Andrews and Gibbons 2005). In addition, many snakes are killed intentionally by drivers because of erroneous preconceptions of their venomous nature (Secco et al. 2014). Given that many species of snakes exhibit a small body size, their carcasses are expected to disappear from the road in less than one or two days, a shorter period than some protocols for monitoring roadkill (Antworth et al. 2005; Langen et al. 2007; DeGregorio et al. 2011; Santos et al. 2011). Therefore, the number of road-killed snakes can be highly underestimated if carcass persistence is not factored into roadkill rate calculations.

The only national estimate of snake roadkill for Colombia was published by Lynch (2012), based on records obtained on a road in Brazil (Monteiro et al. 2011) and a road in the western Andes of Colombia (Vargas-Salinas et al. 2011). That author mentions that mortality in Colombia due to collisions with vehicles could range from 52,600 to 176,660 snakes/yr in the primary road network of the country (10,300 km). However, that estimate is inaccurate because roadkill rates of snakes

vary according to the characteristics of the roads, as well as features associated with the ecosystem bisected by the roads (Forman et al. 2003; van der Ree et al. 2015). Furthermore, estimates of roadkill rates provided by Lynch (2012) for Colombia are based on a conservative approximation of carcass persistence on roads (e.g., 3.5 days; Vargas-Salinas et al. 2011).

In this study, an initial estimate was made of the time that a snake carcass can persist on two roads in Colombia. Then, the relationships between the carcass persistence time and both snake body size and traffic frequency were examined. Finally, the roadkill rate of snakes on a secondary road in the Central Andes of Colombia studied by Quintero-Ángel et al. (2012) was recalculated by taking into account the results obtained here about carcass persistence. This recalculation was made to illustrate the importance of correcting roadkill rates for carcass persistence, and also to improve knowledge of road ecology, specifically, wildlife roadkills in Colombia. With these analyses, we seek to draw attention to the ecological impacts of roads on this country's snake fauna.

Materials and Methods

Study Area and Methodology

This study was conducted in a 4.5-km stretch along two roads located in the department of Quindío, in the Central Andes of Colombia (Fig. 1). One is a secondary road that connects the *Autopista del Café* (a major highway system) with the town of Filandia, which has an average width of 8 m and a traffic frequency of <1,000 vehicles/day. The other is a primary road (*Autopista del*



Fig. 2. Images of some species of snakes present in the study area and used in the field experiments. (A) *Mastigodryas boddaerti*, (B) *Tantilla melanocephala*, (C) *Dipsas sanctijoannis*, (D) *Erythrolamprus epinephelus*, (E) *Leptodeira annulata*, (F) *Oxyrophus petolarius*. Photos by Lina M. Robayo-Palacio (A), Fernando Vargas-Salinas (B–D), Ana María Ospina-L (E), and Wolfgang Buitrago-González (F).

Café) with a traffic frequency of >2,500 vehicles/day. This road is bordered by two margins, each with a width of 8 m. Because the two margins were separated by a median strip, and the field experiments were performed in just one of the margins (see below), the carcasses were assumed to be subjected to the impact of half of the traffic level (i.e., ~1,250 vehicles/day). The minimum distance between the two 4.5-km road segments was 1.5 km; and since the road stretches are close to each other, climatic conditions were assumed to be similar between them.

The temperature in the study area varies between 12 and 18 °C, with 83% mean annual relative humidity, and a mean precipitation of 2,515 mm/yr. The rainfall regime in this area is bimodal, with high precipitation levels during April–May and October–November (CRQ 2010); however, during the sampling period (August 2015–March 2016) summer environmental conditions predominated due to the “El Niño” phenomenon (NOAA

<http://www.noaa.gov/understanding-el-nino>). The landscape surrounding both roads consisted of paddocks, croplands, and some remnants of bamboo (*Guadua angustifolia*) and secondary forest (Serna 2012).

Between August 2015 and March 2016, 81 carcasses of snakes were placed randomly in the two roads under study. Nine snake carcasses were placed on each road spaced 500 m apart. The carcasses were placed on the roads between 0630 and 0700 h, and were monitored continuously throughout the day until 1800 h. The monitoring consisted of direct observations with binoculars at a distance of 50 m to avoid interfering with potential scavenger activity. The time of carcasses persistence and the causes of their eventual disappearance were recorded. A carcass was considered to be no longer detected when it was absent from the asphalt and the ground next to the road, or when the body was fragmented and degraded to a level that became unrecognizable from a potential

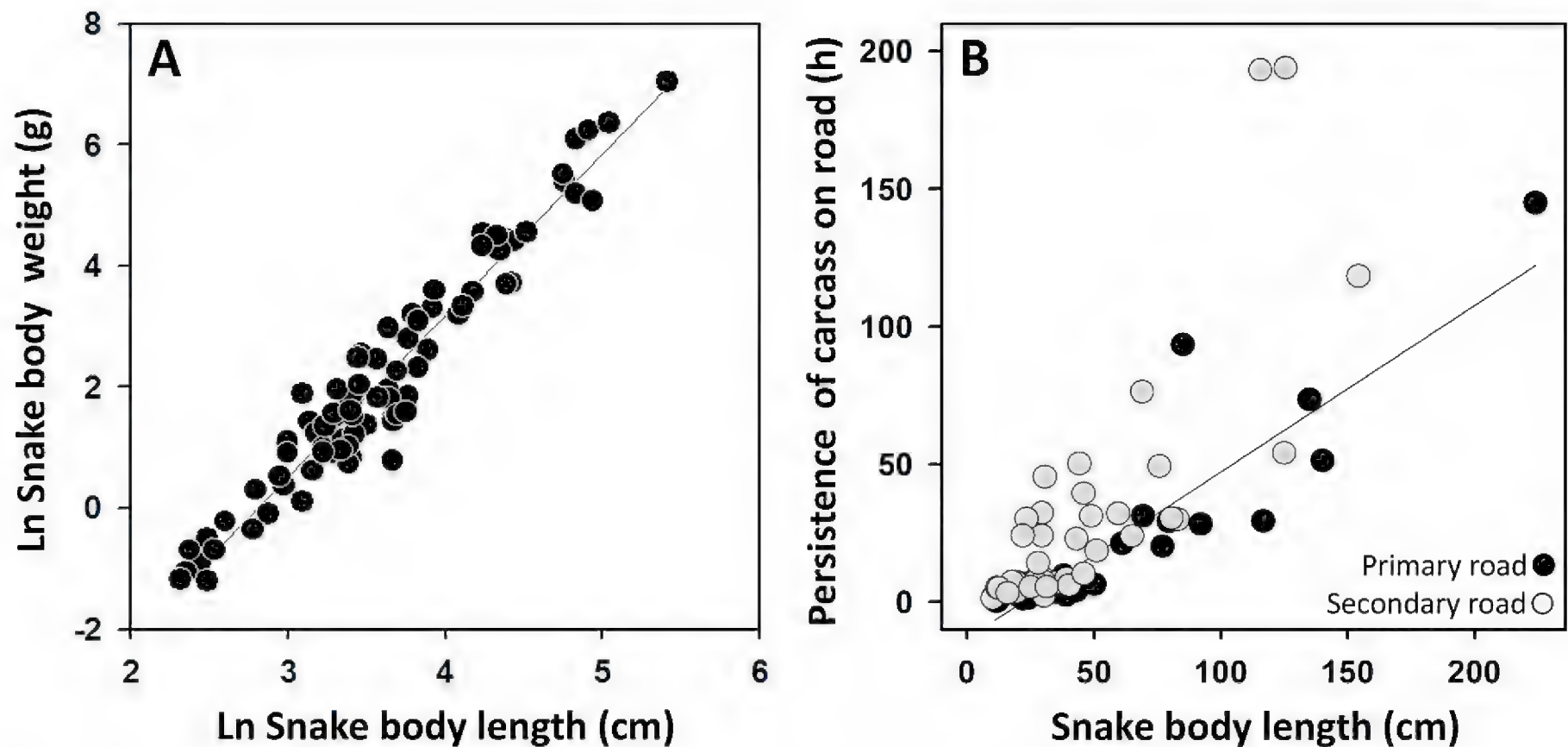


Fig. 3. (A) Relationship between weight and body length (Ln = natural logarithm) of snake carcasses used in this study. (B) Relationship between body length of snake carcasses and their persistence time on two roads with different levels of vehicular traffic.

observer located at the margin of the road. When carcasses disappeared during night (due to scavengers or any other factors), they were assumed to be removed the following morning when the monitoring restarted; in this way we were conservative in our estimates. Snake carcasses used in the present study were obtained from deaths and roadkills collected between April 2015 and March 2016 (Fig. 2; Appendix 1) in various places in the department of Quindío, Colombia. This study only used snakes recently run over with a non-flattened body and with fresh tissues and blood.

Although the carcasses used were taken from different locations in the department of Quindío, the species were locally distributed in the area of monitoring. The carcasses were frozen until the beginning of the field experiment. Individual taxonomic identification of the road-killed snakes was conducted based on prior knowledge of the species (Quintero-Ángel et al. 2012; Vanegas-Guerrero et al. 2016) and taxonomic descriptions in the literature (e.g., Lynch and Passos 2010; Peters and Orejas-Miranda 1970).

Data Analysis

A *t*-test was used to verify that there were no differences in the total length (hereafter, body size) or mass of the carcasses used in the field experiments between the primary and the secondary roads. A high correlation between the body size and weight of the snake carcasses was corroborated using a linear regression analysis; therefore, only the body size of the individual was used for subsequent analysis.

To achieve the first aim of this study, the median value of carcass time persistence for each type of road was estimated. In addition, a Kaplan-Meier estimator

(Therneau and Grambsch 2000) was performed to calculate the probability of a carcass remaining on the road surface (i.e., potentially detectable by a researcher) through time. These probabilities were obtained for the primary and secondary roads separately. For the second objective, an analysis of covariance (ANCOVA) was applied; where the snake body size and type of roadway (primary, secondary) were the explanatory variables, while the carcass persistence time was the response variable. To achieve the third objective, the median persistence time of snake carcasses obtained for the secondary road in the study was used to recalculate the roadkill rate published by Quintero-Ángel et al. (2012). This estimation was reasonable because that study was made in the same secondary road stretch as the current experiments. The new roadkill rate estimate (RRE) was made using the following equation:

$$RRE = \left[\frac{\text{Number of carcass}}{\text{Length of surveyed road stretch (Km)}} \right] \left[\frac{\text{Days of year (365)}}{\text{Monitoring time period (days)}} \right] \left[\frac{\text{Time between samplings (days)}}{\text{Median persistence time (in days) of carcass}} \right]$$

Results

There were no differences in the body size and weight of the carcasses between the primary and secondary roads (body size: $t = 0.283$, $df = 79$, $P = 0.77$; body weight: $t = 0.451$, $df = 79$, $P = 0.653$), and there was a strong positive relationship between the body size and weight of the snake carcasses ($R^2 = 0.73$, $F = 225.135$, $\beta = 0.86$, $P < 0.001$; Fig. 3A).

In this study, 81 carcasses were used which belonged to 14 species distributed in three families: Colubridae (41 individuals), Dipsadidae (39), and Elapidae (1).

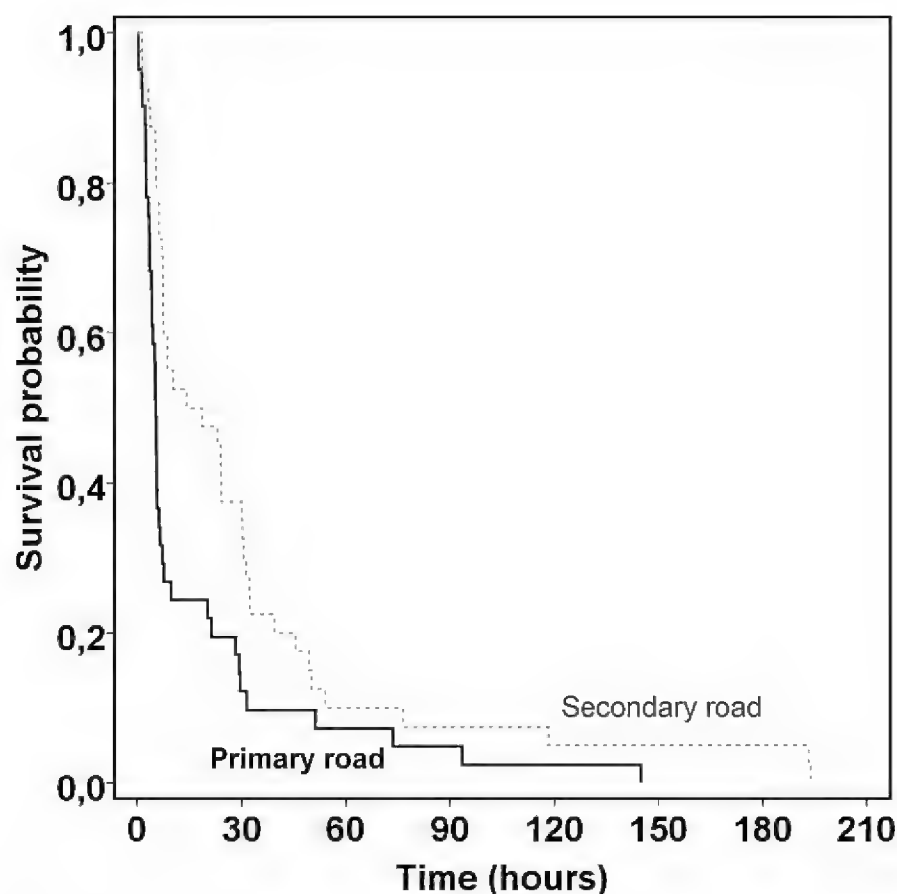


Fig. 4. Survival curves (i.e., probability of persistence on the road) for snake carcasses on primary and secondary roads in the municipality of Filandia, department of Quindío, Central Andes of Colombia. Kaplan-Meier estimates based on a sample size $N = 81$ snake carcasses.

The median persistence time of the snake carcasses on the primary road was 5.20 h (range = 0.26–145.0 h; $N = 41$ individuals), while on the secondary road it was 14.16 h (range = 0.27–193.90 h; $N = 40$ individuals). By combining the data from both types of roadway, the median persistence time of the carcasses was 7.16 h (range = 0.26–193.90 h). The persistence probability dropped substantially beyond 24–48 h for both the primary and secondary roads; in other words, after a snake has been killed, the probability that a researcher would be able to record the carcass on the road is less than 0.25 after, for example, 30 h (Fig. 4). During the daytime, the main cause of carcass disappearance was degradation due to vehicular traffic (65 of the 81 carcasses), and only three carcasses were removed by scavengers (birds). Thirteen other carcasses disappeared at night so it was not possible to determine the cause (see Appendix 1).

Compared to carcasses with a smaller body size, snakes with a larger body size tended to persist longer on the roads (ANCOVA $F = 169.68$, $df = 1$, $P < 0.001$). Although the type of road did not influence the carcass persistence time ($F = 0.124$, $df = 1$, $P = 0.725$), the analysis of covariance revealed that carcass size/type of road interaction had a significant effect on carcass persistence ($F = 11.35$, $df = 1$, $P = 0.001$). If the carcass was small, it tended to persist for the same time on the primary road as on the secondary road; however, when the carcass was large, the difference in persistence time between road types increased, being lower on the primary road compared to the secondary road (Fig. 3B). Using these results of median carcass persistence time on the secondary road, the roadkill rates of snakes for the location reported by Quintero-Ángel et al. (2012),

increased substantially, from 78.8 individuals/km/year to 934.83 individuals/km/year.

Discussion

The estimate of snake carcass persistence time on roads followed a general trend found in similar studies. In North America, South America, and Europe, nearly 50% of carcass disappearance from the roadway occurs during the first 8 to 24 h (DeGregorio et al. 2011; Santos et al. 2011).

Snake carcasses with a larger body size were found to persist longer than carcasses with a smaller body size on the roads. This result is in agreement with Santos et al. (2011), but disagree with the findings of Antworth et al. (2005), DeGregorio et al. (2011), and Hubbard and Chalfoun (2012). The results here differ from those observed by Antworth et al. (2005) possibly because a relatively small range of snake carcass sizes (54.3–136 cm) was used in that study compared with the current study (range: 10.1–224 cm; see Appendix 1). Regarding the results obtained by DeGregorio et al. (2011), these authors placed the carcasses on the roadsides to evaluate only the effect of scavengers on carcass persistence, excluding the effect of traffic. In this study, carcass persistence was mainly determined by the action of vehicular traffic and not by scavengers. Finally, it is possible that Hubbard and Chalfoun (2012) did not find a relationship between carcass body size and persistence time because they used portions of fishes for their field experiments, which do not resemble the consistency of snake carcasses that are covered with scales and include a bone structure (i.e., spinal column). Characteristics such as scales, the presence or absence of hair, and spines on animal bodies have been found to allow corpses to better withstand the passage of vehicles and last longer on roadways (Santos et al. 2011).

The lack of statistical differences in the persistence time of carcasses between the primary and secondary roads might be attributed to the relatively small difference in traffic that could potentially hit the carcasses (~1,250 vehicles/day vs. <1,000 vehicles/day, respectively). Nevertheless, Santos et al. (2011) also found no significant differences on snake carcass persistence among four types of roadways (<1,000; 1,000–4,000; 4,000–10,000; and >10,000 vehicles/day). However, this result could be a sampling artifact, given the relatively small sizes of the snakes used in that study (15–240 g), which may not have allowed the recording of an interaction between the level of traffic and the animal's body size. The present study used snake carcasses varying between 0.3 and 1,147.29 g (see Appendix 1) and found that differences in the persistence of carcasses among roads with a different frequency of traffic emerged with particularly large animals. A similar situation may have occurred in Ratton et al. (2014), where a slight variability in weight (mean = $39.6 \pm$ (SD) 2.1 g) of poultry corpses

(*Gallus domesticus*) may have accounted for the absence of significant differences in the persistence of carcasses between a highway and an unpaved roadway with sparse vehicular traffic.

The recalculation of the snake roadkill rate obtained by Quintero-Ángel et al. (2012) on a secondary road of Central Andes of Colombia, pointed out that including the data of carcass persistence time may increase estimates of snake roadkill significantly; specifically, an 11.86-fold increase. However, the same level of increase should not necessarily be found in other localities or with other organisms. In this regard, recent studies testing previous estimates of roadkills in countries such as Brazil, Spain, United States of America, and Wales, showed a high underestimation in roadkill values (Slater 2002; Loss et al. 2014; Ruiz-Capillas et al. 2015; Santos et al. 2011; Santos et al. 2016).

Because roadkill rate and carcass persistence time can vary significantly between areas with different landscape configurations, weather regimes, and roads, it was not possible to estimate snake roadkill at the country level. The simple extrapolation of the results from the current study area (which is small in proportion to the size of the road network in Colombia) is not adequate. This is especially true for a country like Colombia, which exhibits an intricate topography that is reflected in a high diversity of ecosystems and a concomitant spatial variation of snake diversity (Lynch 2012). Additional studies examining snakes road-killed in different regions and ecosystems of Colombia could help to provide a better estimate of snake mortality for the country (e.g., Payan et al. 2013; Castillo-R et al. 2015; De La Ossa-V and Galván-Guevara 2015; Rincón-Aranguri et al. 2019); however, in those studies carcass persistence was not evaluated.

Finally, according to the results obtained here, in order to accurately estimate the roadkill rate for snakes, and many other small vertebrates, a daily monitoring schedule should be adequate (see Santos et al. 2011). This monitoring frequency is higher than those reported in previous studies from Colombia and other countries. Nevertheless, it is important to remember that this sampling capacity would depend to a large degree on the economic support available.

Conclusion and Perspectives

This study highlights that the problem of road-killed snakes on the road infrastructure of Colombia is greater than previously believed. More data on carcass persistence time obtained from different localities and different types of roads (e.g., paved, non-paved) around the country are necessary for determining an accurate estimate of the number of snakes killed annually in Colombia. Given that collisions may be related to a species population size (Rosen and Lowe 1994; Row et al. 2007; Fahrig and Rytwinski 2009), these results bear important

implications for visualizing the need for conservation plans against roadkills. Snakes are important prey and predators in terrestrial ecosystems (Diller and Johnson 1988; Godley 1982), and for this reason, the impact of roadways on snake populations might even be reflected at the ecosystem level (Forman and Alexander 1998).

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Laura Ximena Cabrera-Casas and Lina Marcela Robayo-Palacio are both graduates from the Biology program at Universidad del Quindío, Colombia. Their research interests are focused on studying inter-specific interactions and their effects on the structure and composition of Neotropical reptile assemblages.



Fernando Vargas-Salinas is an Associate Professor in the Biology program at Universidad del Quindío in Armenia, Colombia, where he is in charge of the academic areas of ecology, animal behavior, and herpetology. Fernando's research interests are focused on the ecological and behavioral aspects of Neotropical vertebrates and their relations with evolutionary processes and conservation biology. For this, he mainly studies acoustic communication and reproductive behavior in anurans, and the diversity of amphibians and reptiles in environments disturbed by the implementation of agricultural activities or the presence of roads and urban complexes.

Appendix 1. Taxonomic identity and associated information of snake carcasses used in the field experiments.

Taxon	Collection date	Place of origin	Body length (cm)	Weight (g)	Cause of disappearance	Period of disappearance
COLUBRIDAE						
<i>Chironius monticola</i>	7/10/2015	Filandia	125	179.5	traffic	day
<i>Chironius monticola</i>	27/05/2015	Buenavista	48.9	13.8	traffic	day
<i>Dendrophidion bivittatus</i>	27/11/2015	Filandia	29.5	2.8	traffic	day
<i>Lampropeltis triangulum</i>	4/10/2015	Filandia	69.3	93.33	traffic	day
<i>Lampropeltis triangulum</i>	13/10/2015	Filandia	69	76	traffic	day
<i>Mastigodryas boddaerti</i>	13/04/2015	Calarcá	79.4	86.43	traffic	day
<i>Mastigodryas boddaerti</i>	24/03/2016	Quimbaya	140	160.8	traffic	day
<i>Spilotes pullatus</i>	4/11/2015	Quimbaya	224	1,147.29	traffic	day
<i>Tantilla melanocephala</i>	12/08/2015	Filandia	26	2.6	traffic	day
<i>Tantilla melanocephala</i>	26/06/2015	Filandia	29.4	5.16	traffic	day
<i>Tantilla melanocephala</i>	10/05/2015	Filandia	43.1	6.4	traffic	day
<i>Tantilla melanocephala</i>	20/08/2015	Filandia	39	2.2	traffic	day
<i>Tantilla melanocephala</i>	15/05/2015	Filandia	27	4.5	traffic	day
<i>Tantilla melanocephala</i>	25/08/2015	Autopista del Café	12	0.61	traffic	day
<i>Tantilla melanocephala</i>	20/08/2015	Autopista del Café	11.5	0.41	traffic	day
<i>Tantilla melanocephala</i>	1/09/2015	Autopista del Café	38.2	6.17	traffic	day
<i>Tantilla melanocephala</i>	19/09/2015	Filandia	27	2.5	traffic	day
<i>Tantilla melanocephala</i>	10/09/2015	Filandia	35.5	6.22	traffic	day
<i>Tantilla melanocephala</i>	10/09/2015	Filandia	33	3.96	traffic	day
<i>Tantilla melanocephala</i>	10/09/2015	Filandia	24	3.5	traffic	day
<i>Tantilla melanocephala</i>	13/02/2016	Filandia	42.4	4.9	traffic	day
<i>Tantilla melanocephala</i>	15/02/2016	Filandia	25	2.5	traffic	day
<i>Tantilla melanocephala</i>	15/02/2016	Filandia	12.5	0.5	traffic	day
<i>Tantilla melanocephala</i>	7/02/2016	Circasia	16	0.7	traffic	day
<i>Tantilla melanocephala</i>	7/11/2015	Autopista del Café	25.1	2.7	traffic	day
<i>Tantilla melanocephala</i>	7/11/2015	Autopista del Café	28.5	3.1	unknown	night
<i>Tantilla melanocephala</i>	7/11/2015	Autopista del Café	10.7	0.5	traffic	day
<i>Tantilla melanocephala</i>	7/11/2015	Autopista del Café	20	2.5	traffic	day
<i>Tantilla melanocephala</i>	10/07/2015	Filandia	39.2	4.19	traffic	day
<i>Tantilla melanocephala</i>	10/07/2015	Autopista del Café	10.5	0.35	traffic	day
<i>Tantilla melanocephala</i>	5/09/2015	Filandia	30.33	6.6	traffic	day
<i>Tantilla melanocephala</i>	5/09/2015	Filandia	25.4	3.87	traffic	day
<i>Tantilla melanocephala</i>	2/09/2015	Autopista del Café	10.1	0.31	traffic	day
<i>Tantilla melanocephala</i>	2/09/2015	Filandia	40.2	4.7	traffic	day
<i>Tantilla melanocephala</i>	29/10/2015	Filandia	30.34	4.61	traffic	day
<i>Tantilla melanocephala</i>	19/10/2015	Filandia	19	1.7	traffic	day
<i>Tantilla melanocephala</i>	6/11/2015	Filandia	12	0.3	traffic	day
<i>Tantilla melanocephala</i>	19/10/2015	Filandia	17.7	0.92	traffic	day
<i>Tantilla melanocephala</i>	19/10/2015	Filandia	23.4	1.85	traffic	day
<i>Tantilla melanocephala</i>	27/02/2016	Filandia	28	2.6	unknown	night
<i>Tantilla melanocephala</i>	17/02/2016	Filandia	13.5	0.8	traffic	day

Roadkill snake persistence in Colombia

Appendix 1 (continued). Taxonomic identity and associated information of snake carcasses used in the field experiments.

Taxon	Collection date	Place of origin	Body length (cm)	Weight (g)	Cause of disappearance	Period of disappearance
DIPSADIDAE						
<i>Atractus</i> cf. <i>melanogaster</i>	20/08/2015	Filandia	23	4.18	traffic	day
<i>Atractus</i> cf. <i>melanogaster</i>	20/08/2015	Filandia	38.8	4.24	traffic	day
<i>Atractus</i> cf. <i>melanogaster</i>	5/09/2015	Autopista del Café	30.6	3.4	traffic	day
<i>Atractus</i> cf. <i>melanogaster</i>	15/11/2015	Filandia	30	5	traffic	day
<i>Atractus</i> sp.	19/08/2015	Filandia	20	3.02	traffic	day
<i>Atractus</i> sp.	20/01/2015	Filandia	30	2.32	traffic	day
<i>Atractus</i> sp.	5/09/2015	Filandia	19.5	1.45	unknown	night
<i>Atractus</i> sp.	30/02/2016	Filandia	27.5	7.2	traffic	day
<i>Atractus</i> sp.	4/12/2015	Filandia	22	6.7	traffic	day
<i>Atractus</i> sp.	7/03/2016	Filandia	31.4	7.8	traffic	day
<i>Atractus</i> sp.	4/09/2015	Filandia	32	12.78	traffic	day
<i>Atractus</i> sp.	30/08/2015	Filandia	43.1	16.42	unknown	night
<i>Clelia</i> sp.	23/11/2015	Filandia	75.8	90.2	traffic	day
<i>Clelia</i> sp.	8/11/2015	Filandia	115.5	249.7	traffic	day
<i>Clelia</i> sp.	4/12/2015	Filandia	125.5	443.1	traffic	day
<i>Clelia</i> sp.	17/03/2016	Quimbaya	154.3	581.2	unknown	night
<i>Clelia</i> sp.	17/02/2016	Filandia	135	520.06	traffic	day
<i>Dipsas</i> cf. <i>sanctijoannis</i>	20/09/2015	Filandia	85	83.1	unknown	night
<i>Dipsas</i> cf. <i>sanctijoannis</i>	28/09/2015	Filandia	77	70.3	unknown	night
<i>Dipsas</i> cf. <i>sanctijoannis</i>	22/07/2015	Filandia	83	41.69	traffic	day
<i>Dipsas</i> cf. <i>sanctijoannis</i>	19/10/2015	Filandia	80.5	40.71	scavenger	day
<i>Dipsas</i> cf. <i>sanctijoannis</i>	11/10/2015	Filandia	65.1	35.94	traffic	day
<i>Dipsas</i> cf. <i>sanctijoannis</i>	6/11/2015	Filandia	46	10.2	unknown	night
<i>Dipsas</i> cf. <i>sanctijoannis</i>	12/02/2016	Circasia	61	28.4	unknown	night
<i>Dipsas</i> cf. <i>sanctijoannis</i>	5/03/2016	Filandia	92	95.5	traffic	day
<i>Dipsas</i> cf. <i>sanctijoannis</i>	10/09/2015	Filandia	116.6	222.33	traffic	day
<i>Erythrolamprus epinephelus</i>	7/06/2015	Filandia	38	19.65	traffic	day
<i>Erythrolamprus epinephelus</i>	21/05/2015	Filandia	38	7.1	unknown	night
<i>Erythrolamprus epinephelus</i>	20/08/2015	Filandia	50.2	27.46	traffic	day
<i>Erythrolamprus epinephelus</i>	25/09/2015	Filandia	16.3	1.36	traffic	day
<i>Erythrolamprus epinephelus</i>	10/07/2015	Filandia	35.4	11.73	unknown	night
<i>Erythrolamprus epinephelus</i>	2/09/2015	Filandia	44.2	24.66	traffic	day
<i>Erythrolamprus epinephelus</i>	29/10/2015	Filandia	26.8	4.82	scavenger	day
<i>Erythrolamprus epinephelus</i>	7/11/2015	Filandia	51	36.6	traffic	day
<i>Erythrolamprus epinephelus</i>	30/11/2015	Filandia	46	22.1	unknown	night

Appendix 1 (continued). Taxonomic identity and associated information of snake carcasses used in the field experiments.

Taxon	Collection date	Place of origin	Body length (cm)	Weight (g)	Cause of disappearance	Period of disappearance
<i>Erythrolamprus epinephelus</i>	27/12/2015	Filandia	40	9.7	traffic	day
<i>Oxyrhopus petolarius</i>	12/11/2015	Caicedonia	29.5	2.09	traffic	day
<i>Oxyrhopus petolarius</i>	29/10/2015	Filandia	59.5	24.44	scavenger	day
<i>Leptodeira annulata</i>	7/03/2016	Armenia	31.2	12.1	unknown	night
ELAPIDAE						
<i>Micrurus mipartitus</i>	4/04/2015	Quimbaya-Filandia road	22	1.12	traffic	day



Effect of vegetation and abiotic factors on the abundance and population structure of *Crocodylus acutus* (Cuvier, 1806) in coastal lagoons of Colima, Mexico

^{1,2,5}Sergio Aguilar-Olguín, ^{2,5}María Cruz Rivera-Rodríguez, ³Helios Hernández-Hurtado,
⁴Ricardo González-Trujillo, and ^{1,*}María Magdalena Ramírez-Martínez

¹Universidad de Guadalajara, Centro Universitario de la Costa Sur, Av. Independencia Nacional 151, 48900, Autlán de Navarro, Jalisco, MÉXICO
²UMA Centro Ecológico de Cuyutlán “El Tortugario,” Av. López Mateos S/N, Poblado de Cuyutlán 28350, Armería, Colima, MÉXICO ³Universidad de Guadalajara, Centro Universitario de la Costa, Av. Universidad 203, Ixtapa, 48280, Puerto Vallarta, Jalisco, MÉXICO ⁴Centro de Investigación para los Recursos Naturales, Antigua Normal Rural de Salaces, C. P. 33941, López, Chihuahua, MÉXICO ⁵Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Autopista Colima-Manzanillo Km 40, La Estación, C. P. 28930, Tecmán, Colima, MÉXICO

Abstract.—Crocodile populations are affected by their environment, and disturbance of that environment leads to changes in their physiology and behavior. Using nocturnal spotlight counts, the influences of vegetation type and several abiotic factors on populations of *Crocodylus acutus* were evaluated in Colima, Mexico. Six interconnected lagoons with a known presence of crocodiles were selected, and the largest (Laguna de Cuyutlán) was divided into four sections. Differences in crocodile density and size classes among these lagoons were determined, and the effects of abiotic factors and vegetation type on the density and distribution of crocodiles were identified. Salinity could influence the crocodile populations, since low crocodile densities were observed in lagoons with high salinity. Average densities of crocodiles of 0.2–8.3 individuals/km and 0–5.9 ind/km were recorded during the rainy and dry seasons, respectively. The average densities of crocodiles of size classes I, II, and III ranged from 0.3–1.7 ind/km, whereas those of size classes IV and V ranged from 0.1–1.8 ind/km. Population densities of crocodiles were associated with factors such as salinity (<1%), and since the hatchlings and juveniles are the most vulnerable to conditions of high salinity, they are drawn to sites of lower salinity, such as those with aquatic and mangrove vegetation. This suggests that *C. acutus* can find refuge and food in the mangrove vegetation and water at ambient temperatures of 3.9–6.3 °C. Variations observed in both the water and ambient temperatures probably did not affect the normal thermoregulation processes of the crocodiles, since they can adopt a strategy of thermoconformity in response to even minor variations in temperature. There were significant differences ($P < 0.05$) among the lagoons in terms of salinity, aquatic and mangrove vegetation, and water and ambient temperatures. The coastal lagoons of Colima provided suitable habitats for crocodile distribution, but increased salinity led to the movement of crocodiles towards areas supplied with freshwater.

Keywords. American Crocodile, depth, habitat, Pacific, salinity, wetlands

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Introduction

The American Crocodile, *Crocodylus acutus*, has the widest species distribution of its genus on the American continent. It is found from southern Florida to Colombia on the Atlantic side, and from Mexico to Peru on the Pacific side of the continent (Brandt et al. 1995). The species inhabits shallow waters and, while it prefers freshwater, the American Crocodile can tolerate high salinities and use saltwater routes to move to reproduction, feeding, nesting, and refuge areas (Lang

1987; Thorbjarnarson et al. 2006; Mazzotti et al. 2007; Cupul-Magaña 2012). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists *C. acutus* in Appendix II, the International Union for the Conservation of Nature (IUCN) lists the species as Vulnerable, and the Official Mexican Norm 059-SEMARNAT-2010 considers it a species subject to special protection (DOF 2010).

Large specimens can be found at great depths in Costa Rica, and high water salinity levels lead to a greater dispersion of the animals (Mauger et al. 2012).

Correspondence. ^{1,2,5}zanyya@hotmail.com; ^{2,5}mary_cruz@live.com.mx; ³hjh0474@hotmail.com; ⁴ecologia.rgt@gmail.com;
^{1,*}maleni.ramirez@gmail.com

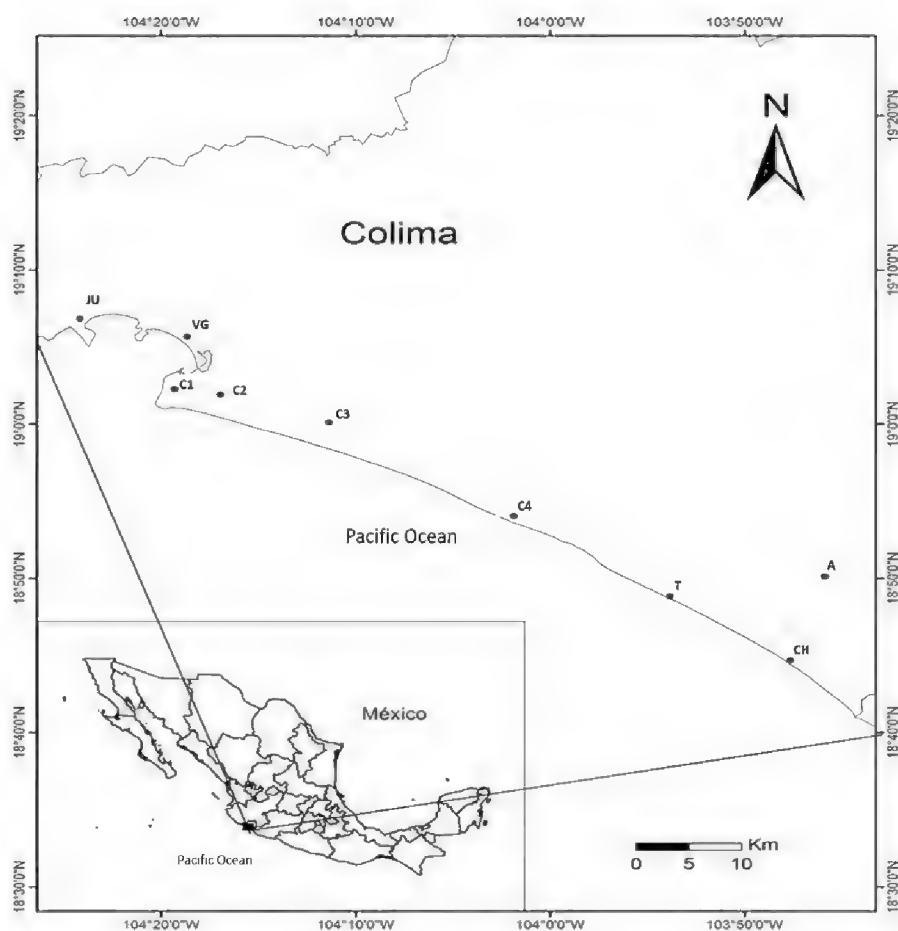


Fig. 1. Selected sites in the study area. Acronym definitions and characteristics of the sites are given in Table 1.

Relationships between low crocodile densities and increased water temperature, high salinity, and poor vegetation cover have been reported in Ecuador (Carvajal et al. 2005). However, Cherkiss et al. (2011) did not find a relationship between water or air temperature and crocodile densities in Florida, although these authors did observe that all crocodile size classes preferred protected canals and ponds of low salinity surrounded by vegetation. The distribution of American Crocodiles in Colombia is influenced mainly by resource availability (Balaguera and González 2008). It is therefore important to determine the relationships between these variables and crocodile densities in Mexico, compared to other populations of this species.

While García-Grajales and Buenrostro-Silva (2014) did not analyze salinity values on the coast of Oaxaca, Mexico, these authors determined that high crocodile densities were associated with mangrove vegetation since the crocodiles preferred to stay hidden among the mangrove roots. This finding coincides with previous reports from Jalisco and Veracruz, where the density of crocodiles *C. acutus* (Cupul-Magaña 2012) and *Crocodylus moreletii* (González-Trujillo et al. 2014) decreased in modified habitats unless a strip of vegetation was present near the water bodies. In Nayarit, Mexico, Hernández-Hurtado et al. (2011) reported high densities of all sizes of *C. acutus*, which were associated with sites of low salinity, depths exceeding 1 m, and six types of vegetation, conditions in which the crocodiles presented greater dispersion and benefited from a higher availability of food and shelter.

The coastal area of Colima, Mexico, has undergone changes due to extensive construction of infrastructure. Over a period of 13 years, 145.40 ha of mangrove have been lost, out of a total area of 494.02 ha, which

has modified the physical and biological structure of the coastal ecosystems (Jiménez-Ramón et al. 2016). While it is likely that a consequent modification of the crocodile habitat could have occurred, the biotic and abiotic variables related to the density and distribution of American Crocodile populations that inhabit the area are unknown. Therefore, the objective of the present study was to evaluate the state of the crocodile populations in Colima, Mexico, and to determine which habitat variables influence the abundance, structure, and distribution of this species. A greater crocodile abundance was expected at lower salinities, as well as a greater density of adult crocodiles in areas with higher temperatures, and higher numbers of hatchlings and juveniles in areas with a greater presence of aquatic vegetation.

Materials and Methods

Study area. The coastal zone of the state of Colima, which comprises the study area, presents annual temperatures ranging between 20 and 28 °C and mean annual precipitation of 947.8 mm. The rainy season begins in June, and precipitation events can be torrential, especially at the end of August and the beginning of September, while the rains begin to decrease in November (Arévalo et al. 2016). This region supports vegetation communities of low elevation dry deciduous forest, scrub forest, and mangrove forest. Lagoons with a known presence of crocodiles were selected for this study, based on information provided by the local environmental authorities and communities established close to each lagoon. Six lagoons were selected, with the largest (Laguna de Cuyutlán) divided into four sections that communicated directly with each other (see Fig. 1 and Table 1 for lagoon abbreviations). The dominant vegetation found in the selected lagoons is mangrove forest comprising the species *Rhizophora mangle* and *Laguncularia racemosa*, with secondary vegetation such as *Acacia* sp., and floating vegetation such as *Typha domingensis* and *Echhornia crassipes*. It should be noted that some lagoons are permanently connected to the ocean (JU, C1, C2, C3), while others are connected for only part of the year (VG, T, CH), and some are completely disconnected from the ocean (C4 and A).

Sampling data. Night visits were made twice during the rainy season (once in June 2014 and once in November 2015) and twice during the dry season (once in March 2015 and once in May 2016). The nocturnal spotlight technique, as proposed by Messel et al. (1981) and Thorbjarnarson et al. (2000), was used to determine the relative population density, reported as individuals per km (ind/km). From a 25 hp outboard motor boat, either a 500-lumen headlamp or a handheld torch was used to illuminate the water surface, the lagoon edges, below the mangrove vegetation, and the emergent vegetation. The individuals counted were grouped into size classes I to V

Table 1. Identification key of study sites and environmental variable value averages for each site during the two seasons. **Ta:** water temperature; **Tai:** ambient temperature; **Sal:** salinity; **P:** depth.

Key	Lagoon/Estuary	Latitude	Longitude	Sal (psu)	Ta (°C)	Tai (°C)	P (m)
JU	Juluapan	19°06'50"	104°24'23"	24.9	28.7	25.9	1.9
VG	Valle de las Garzas	19°05'17"	104°18'20"	2	28.9	27	1.3
C1	Cuyutlán Vaso I	19°02'13"	104°19'18"	32.3	28.8	25.4	1.6
C2	Cuyutlán Vaso II	19°01'12"	104°15'30"	32.1	28.2	25.4	1.1
C3	Cuyutlán Vaso III	19°00'15"	104°12'53"	30.1	31.3	28.3	1.1
C4	Cuyutlán Vaso IV	18°54'05"	104°01'53"	0.5	30.4	27.1	0.8
T	Tecuanillo	18°48'49"	103°53'52"	0.5	26.4	25.4	1.4
CH	Chupadero	18°45'01"	103°48'08"	0.6	29.3	24.8	1.4
A	Amela	18°50'21"	103°45'49"	0.3	31.2	27.4	3.5

according to total body length, which was estimated by calculating the distance from the base of the eyes to the end of the snout of the crocodile and multiplying by ten (Platt and Thorbjarnarson 2000).

Temperature and salinity were recorded at every km traveled on the lagoons. These parameters were measured with a multiparametric PCSTestr35 (OAKTON, Vernon Hills, Illinois, USA), with a salinity meter accuracy of $\pm 1.0\%$ FS and automatic temperature compensation. Depth was measured with an aluminum leveling rod 10 m in length, graduated in centimeters. Vegetation type was the only biotic factor recorded; each lagoon was visited during the day in order to describe the species of flora present, which were grouped into four vegetation types based on the classification by Ramírez-Delgadillo and Cupul-Magaña (1999). The distance covered by each vegetation type along the shore of each lagoon for each km traveled was measured with a GPS brand Garmin model e-trex 10, in order to determine a relationship between the vegetation data and the presence of crocodiles.

Data analysis and statistics. To determine the degree of association between crocodile density, abiotic factors (water temperature, ambient temperature, depth, and salinity), and vegetation, a non-metric multidimensional scaling analysis (NMDS) was performed in the program PAST version 3.08 (Hammer et al. 2001), using the average value of each variable obtained in each selected lagoon. A dissimilarity matrix was obtained using the Bray-Curtis coefficient (Hammer et al. 2001). This analysis creates a stress coefficient, for which a value below 0.1 indicates that the groups formed differ in their compositions. To corroborate the groups obtained, a dendrogram was created using the Bray-Curtis coefficient (Hammer et al. 2001). This dendrogram provided a correlation coefficient which indicated the similarity among the lagoons, with values close to 1 indicating greater similarity.

Differences in crocodile density between seasons, lagoons, and size classes were estimated with a Kruskal-Wallis test ($P < 0.05$). This same analysis was used to evaluate differences in crocodile densities between the lagoons that were connected to each other, and between lagoons that were either always connected

to the ocean, connected for only part of the year, or completely disconnected from the ocean. Differences between abiotic factors per season and per lagoon were evaluated with a Kruskal-Wallis test ($P < 0.05$), and a Tukey test ($P < 0.05$) was used to identify where differences occurred. This same analysis was used to evaluate differences in vegetation per type and per lagoon. All of these analyses were performed using the software package PAST version 3.08 (Hammer et al. 2001).

Results

The study was conducted in a total of six lagoons that collectively represented a surface area of approximately 9,545 ha, and were located along 76.6 linear km of the coastline of Colima state. Significant differences were found in the crocodile densities in these lagoons in both seasons ($P = 0.0001$); the Tukey test indicated that the differences occurred between the lagoons with greater crocodile density (A and VG) and those with lower density (C1, C2, and T). The NMDS analysis and the dendrogram integrated the data from both seasons and generated a stress coefficient of 0.06 and a correlation coefficient of 0.92. There was a separation between the lagoons connected to the ocean (JU, C1, C2, and C3) as one group, and those that were either not connected to the ocean or only connected for part of the year (VG, CH, and T) as another group (Fig. 2). However, the dendrogram showed that lagoon C4 was similar to the group of lagoons not connected to the ocean, while lagoon A was dissimilar to all the others (Fig. 3).

Crocodiles were observed at the nine study sites during both seasons. Relative frequencies ranged from 0.2–8.3 ind/km and 0–5.9 ind/km during the rainy and dry seasons, respectively (Fig. 4). There were significant differences in density in the A and CH lagoons during the two periods ($P = 0.0001$), but no differences were found between size classes ($P = 0.4$).

The density of individuals in the lagoons connected to the ocean (JU, C1, C2, C3) was lower than in those not connected to the ocean or connected for only part of the year ($P = 0.006$). Densities of 0 to 1 ind/km in size classes I, II, and II, and of 0 to 0.4 ind/km in size classes IV and V, were recorded in the lagoons connected to the ocean for

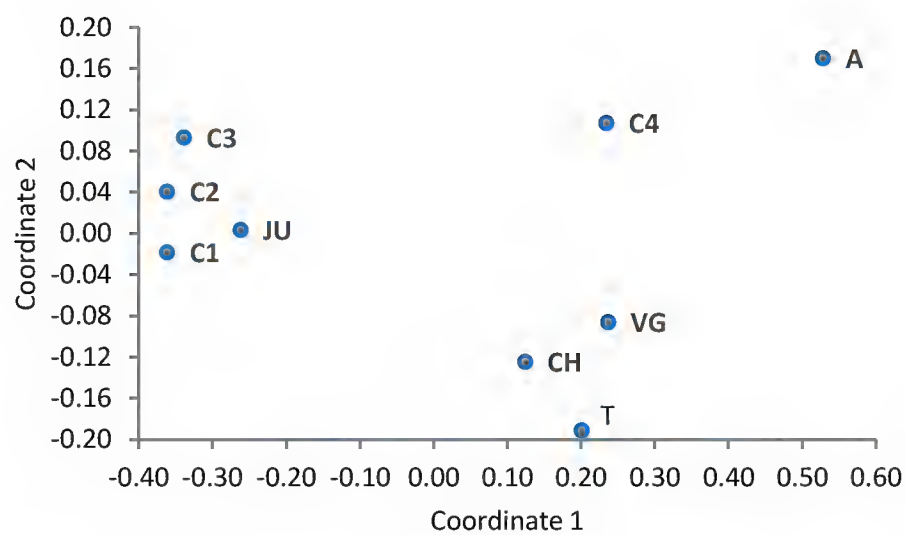


Fig. 2. Non-metric Multidimensional Scaling (NMDS) analysis showing the formation of two groups, by taking into account the crocodiles observed, water salinity, temperature, depth, and the four vegetation types present. Acronym definitions and characteristics of the sites are given in Table 1.

only part of the year (Table 2). Eight species of flora were identified on the shores of the sampling sites, all of which were included in the vegetation classification proposed by Ramírez-Delgadillo and Cupul-Magaña (1999). *Rhizophora mangle* and *L. racemosa* were classified as mangrove forest species, *T. domingensis* and *E. crassipes* as aquatic vegetation, *Acacia* sp. and *Guazuma ulmifolia* as secondary vegetation, and *Batis maritima* and *Cocos nucifera* as pasture-agricultural vegetation. Of the 78.5 linear km traveled, mangrove vegetation was present in eight of the nine lagoons, representing an overall presence of 72%. Site A did not present mangrove vegetation; however, 61% of the banks of this lagoon presented aquatic vegetation and 39% presented secondary vegetation. Of the total crocodile population, 55.4% was recorded in the lagoons where mangrove vegetation occurred, while the remaining crocodiles were recorded in aquatic vegetation (Table 3). There were significant differences in crocodile density between vegetation types ($P = 0.003$). The Tukey test showed that the mangrove vegetation differed significantly ($P < 0.05$) in this regard from the other vegetation types.

Salinity ranged from 0.2–27‰ during the rainy season, and from 0.4–35.3‰ during the dry season. There were significant differences in this parameter between the two seasons ($P = 0.001$) and between the averages of lagoons ($P = 0.0002$); and differences were also detected ($P < 0.05$) between the lagoons connected to the ocean (JU,

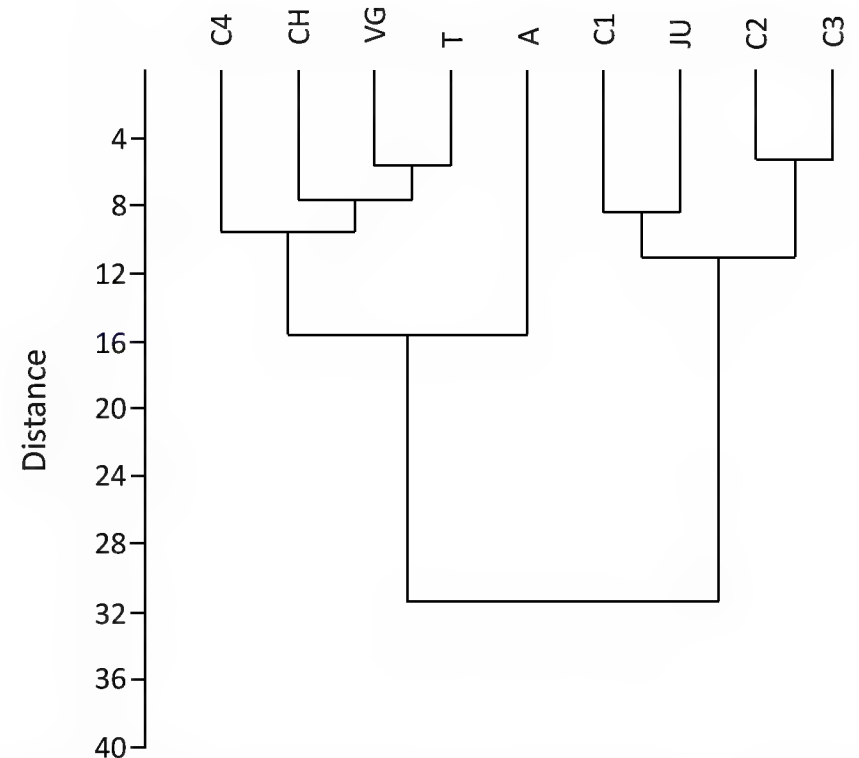


Fig. 3. Dendrogram considering the crocodiles observed, water salinity, temperature, depth, and the four vegetation types present. Acronym definitions and characteristics of the sites are given in Table 1.

C1, C2, C3) and those that were not connected to the ocean (VG, C4, T, CH, A). Water temperature ranged from 25.8–32.1 °C during the rainy season, and from 27.9–31.8 °C during the dry season, with a significant difference found between the two seasons ($P = 0.0002$). The ambient temperature ranged from 22.1–29.3 °C during the rainy season, and from 26.3–28.2 °C during the dry season, also with a significant difference between the two seasons ($P = 0.0002$). The depth range was 0.9–3.5 m during the rainy season and 0.6–3.6 m during the dry season; but there was no significant difference between the two seasons ($P = 0.7$) in this regard, although there were differences ($P = 0.0005$) in depth between lagoons. Lagoon A differed significantly ($P < 0.05$) from the other lagoons (JU, VG, C1, C2, C3, C4, T, CH).

Discussion

Density

The crocodile density ranges found in this study (0–8.3 ind/km) during the two seasons in the nine lagoons can be considered low. This is due to the influences of salinity, ambient temperature, water temperature, and

Table 2. Crocodile population structure expressed as relative density (individuals/km) and percentages (%) for each lagoon, in the two study seasons. **Only eyes** indicates individuals for which the size could not be determined.

Lagoon	Size classes											
	I		II		III		IV		V		Only eyes	
	I/km	%	I/km	%	I/km	%	I/km	%	I/km	%	I/km	%
JU	0	0	0.9	22.4	0.7	15.3	1.1	34.1	0.3	10.6	0.6	17.6
VG	0.5	7.2	1.8	26.4	1.7	24.1	0.8	12	0.4	6	1.7	24.1
C1	0	0	0.1	1	0	0	0	0	0	0	0	0
C2	0.3	35.3	0.2	29.4	0.3	35.3	0	0	0	0	0	0
C3	0.2	7	1	32.8	0.7	21.5	0.6	20.4	0.4	11.3	0.2	7
C4	0.6	27	0.6	29.1	0.5	22.7	0.3	12.1	0.1	4.3	0.1	5
T	0	0	0	0	0	0	1.8	1	0	0	0	0
CH	1	30.3	0.3	7.9	1	31.6	0.4	13.2	0.4	11.8	0.2	5.3
A	0.9	13	0.4	5.7	1	15	1.8	26.8	1.5	22	1.2	17.5

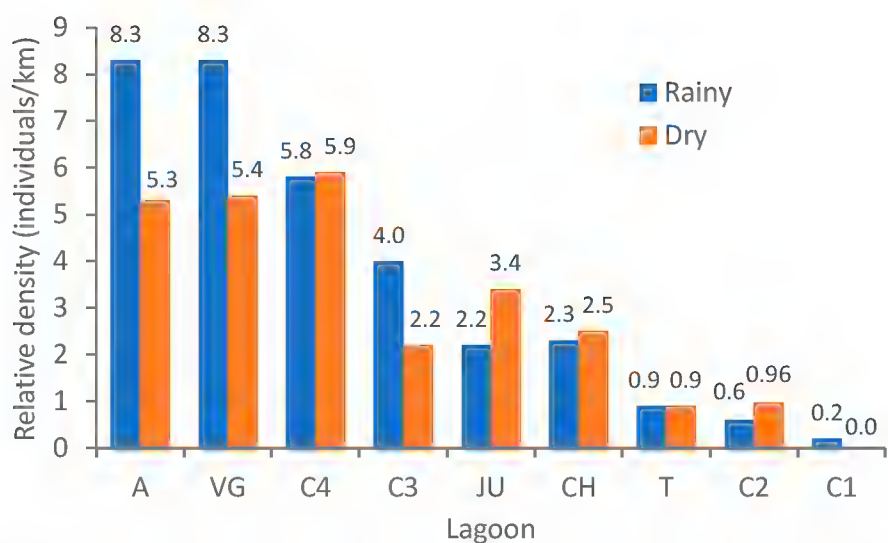


Fig. 4. Average relative *Crocodylus acutus* density in the studied lagoons during the two (rainy and dry) study seasons. Acronym definitions and characteristics of the sites are given in Table 1.

the mangrove vegetation, which varied depending on the connection with the sea in lagoons JU, C1, C2, and C3. This density level can be compared to four other similar studies in different parts of Mexico.

In Chamela-Cuixmala, Jalisco, García et al. (2010) recorded 20.4–32.5 ind/km, classifying this density as high and attributing it to variables such as water depth (0.5 m) and mangrove vegetation cover.

In Boca Negra, Jalisco, Cupul-Magaña et al. (2002) reported 51.2 ind/km and classified this density as high, attributing it to the fact that the area provided the crocodile population with refuge and feeding areas through the presence of mangrove vegetation.

In Palma Sola, Oaxaca, García-Grajales and Buenrostro-Silva (2014) reported a density of 70 ind/km, classifying this value as high and attributing it to the presence of the mangrove vegetation which provided an important refuge area for the crocodiles.

In San Blas, Nayarit, Hernandez-Hurtado et al. (2011) reported densities of 0.36–4.31 ind/km, which were within the ranges found in this study, with the highest densities found in sites with depths greater than 1 m, slow currents, low salinities (4.92–11.03%), high elevations, and six vegetation types, including mangrove swamp.

Thorbjarnarson et al. (2006) designated an area of 22,790 ha as a bioregion of the coast of Colima and Jalisco. They considered the vegetation type as an important variable and estimated a population of between 500 and 1,000 crocodiles. That assessment does not coincide with the results of this study, since in less than half of that area (9,945 ha belonging to the coast of Colima), 506 crocodiles could be observed. However, the difference may be due to the fact that the previous study did not include any of the specific lagoons examined in the present study.

Population Age Structure

The age structure observed in the present study (Table 2) suggests that the populations of crocodiles in Colima are in good health, since the highest percentage of the population belonged to classes I, II, and III, which are classified as juveniles and subadults (Seijas 2011). According to Calverley and Downs (2014), a population structure where the largest number of crocodiles is represented by juveniles and subadults can indicate a positive trend, whereas a population where the largest number of crocodiles is adults indicates a population in decline. In this study, fewer size class III crocodiles were found in lagoons JU, VG, C4, and C3 (Table 2), probably due to dispersion behavior (Cedeño and Pérez 2010; Hernández-Hurtado et al. 2011; García-Grajales and Buenrostro-Silva 2014). This suggests that size class IV and V crocodiles dispersed towards the vegetation because of their territorial behavior, and remained there in refuge without moving towards the open areas of the lagoon (Carvajal et al. 2005; Balaguera and González 2008).

Vegetation Types

In this study, mangrove was the predominant vegetation and it was where the highest percentage of crocodiles was recorded. This suggests that *C. acutus* finds refuge and food in the mangrove vegetation in Veracruz. González-Trujillo et al. (2014) reported the density

Table 3. Linear extents (km) and percentages of vegetation types found in each lagoon.

Lagoon	Mangrove km (%)	Aquatic vegetation km (%)	Secondary vegetation km (%)	Pasture-agriculture km (%)
JU	6 (100)	0	0	0
VG	1.5 (50)	0	0	1.5 (50)
C1	6 (100)	0	0	0
C2	15.5 (97)	0	0	0.3 (3)
C3	16 (100)	0	0	0
C4	4 (67)	2 (33)	0	0
T	0.564 (100)	0	0	0
CH	7 (100)	0	0	0
A	0	11 (61)	7 (39)	0

of *Crocodylus moreletii* was related to vegetation type, with mangrove hosting the largest number of crocodiles and attributed this to the vertical structure of the mangrove, which provides suitable shelter. For *C. acutus* in Oaxaca, García-Grajales and Buenrostro-Silva (2014) recorded the largest number of crocodiles in the mangrove vegetation and attributed this to the fact that crocodiles prefer to remain hidden among the roots of the mangroves. In Ecuador, Carvajal et al. (2005) stated that, in addition to providing refuge for the crocodiles, the mangrove vegetation also provides food resources in the form of invertebrates and vertebrates that also inhabit the mangrove.

However, in Florida, Cherkiss et al. (2011) report that mangrove is the habitat used least by *C. acutus* crocodiles, since the largest number of individuals was observed in artificial ponds, canals, and streams, which offer more protection and this species can easily adapt to anthropogenically created or transformed habitats. In the present study, the Amela (A) lagoon is the largest and has the most abundant aquatic vegetation where the highest densities of crocodiles were recorded.

Abiotic Factors: Salinity, Connection to Ocean, Depth, and Temperature

Salinity was an important factor in crocodile abundance in the different water bodies. The lagoons with the highest salinity formed one group and those with lowest salinity formed another group (Table 1; Figs. 2 and 4). Salinity could negatively affect the crocodile populations, particularly the young (classes I and II) and juveniles (class III), because high salinity causes the individuals of this species to dehydrate, unlike *C. porosus* in Australia which has two glands in the palate that allow it to expel excess salt (Richards et al. 2004). The salinity-sensitivity of this species was reflected in crocodile densities, since the lowest values were recorded in the lagoons with high salinity (Fig. 3). Hernández-Hurtado et al. (2011) also reported a decrease in crocodile density when salinity increased (24.76–35.85%). An increase in crocodile density was observed here from the northern (C1) to the southern (C4) sites sampled within the Cuyutlán Lagoon (C1, C2, C3, and C4). At the latter site, salinity had decreased to concentrations of 0.4–0.7‰ and juvenile and adult crocodiles (classes I, II, and III) were dominant (Table 2). This could be due to the fact that hatchlings and juveniles are the most vulnerable to high salinity concentrations, which would prompt them to seek out sites of lower salinity (Richards et al. 2004).

Dispersion of crocodiles towards different areas in search of freshwater has been reported (Dunson and Mazzotti 1989). A preference of crocodiles for areas with salinity below 1‰ has also been observed (Espinosa et al. 2012). The effect of salinity on crocodiles in classes I and II could be fatal, since hatchlings and juveniles have little tolerance for high salinity (Lang 1987). While some

of the highest salinities in this study were observed at lagoon C2, a large number of hatchlings were recorded there (Fig. 3). However, these animals would die rapidly due to dehydration if they did not move to sites with lower salinity; and this was reflected in the relative densities of classes IV (0 ind/km) and V (0.5 ind/km) [Richards et al. 2004; Hernández-Hurtado 2010].

While lagoon JU was permanently connected to the ocean, crocodiles of all classes, other than class I, were observed there, and they were also observed very close to the mouth of the Miramar Creek. The crocodiles in size classes I, II, and III can avoid the high salinity of the lagoon by finding refuge up this freshwater creek during the rainy season, where they can reach adult sizes. Individuals of *C. acutus* have been reported up this creek (at elevations up to 1,220 m asl) between Guerrero and Oaxaca (Casas-Andreu et al. 1990).

Temperature variations ranged from 3–6 °C (Table 1) in the two study seasons. Carvajal et al. (2005) reported that high temperatures (40 °C) influenced the distribution of crocodiles, which preferred lower temperatures; whereas Espinosa et al. (2012) reported that low temperatures (14.42 °C) influenced the distribution of crocodiles, which were observed in areas with higher temperatures. Lang (1987) reported a positive correlation between water temperature and crocodile density, as water and air temperatures influence the body temperature of crocodiles with implications for their physiological processes. The relatively minor temperature variations observed during this study probably did not affect the normal crocodile thermoregulation processes. This could be due to the fact that crocodiles living in environments with little temperature variability adopt a strategy of thermoconformity. In order to control this physiological process, the crocodiles spend a large part of the day and night in the water regulating their body temperature, a process in which water depth can play an important role (Campos and Magnusson 2012; Grigg et al. 1998; Lang 1987).

The depths observed in the present study ranged from 0.6–1.92 m (Fig. 3), with significant differences in depth among the lagoons. Lagoon A differed significantly from the others, presenting both the greatest depths and highest crocodile density. However, the depths recorded in all of the lagoons were sufficient for the crocodiles to move, reproduce, and feed (Fujisaki et al. 2009). Calverley and Downs (2014) stated that variations in depth could affect the number and distribution of crocodiles. Hernández-Hurtado et al. (2011) recorded the greatest number of crocodiles at depths of over 1 m, which corroborated the finding by Campbell et al. (2010) of a high correlation between water depth and the distance traveled by crocodiles towards feeding areas. Fujisaki et al. (2009) reported that depth had a direct influence on adults during the reproductive period, since both males and females seek water depths of over 1 m in order to mate.

Lagoon A had no similarities with any of the

other lagoons during both seasons. This lagoon was characterized by having mostly aquatic vegetation and abiotic factors that remained rather constant, with water temperature variations of 1 °C, air temperature variations of 2 °C, as well as the greatest depths, and freshwater available throughout the year. The distribution and state of health of the crocodiles at this location were dictated by the low variation in the abiotic factors and aquatic vegetation that provided refuge and food. Moreover, in this case, intraspecific relationships had a greater influence on the population (Lang 1987; Grigg et al. 1998; Balaguera and González 2008; Cherkiss et al. 2011).

Conclusions

In the present study, the abiotic and biotic factors evaluated, including salinity, ambient temperature, water temperature, and mangrove vegetation, were found to have close relationships with the population dynamics of the crocodiles in the State of Colima, and changes in these variables may influence the distribution of *C. acutus* populations. High salinities may restrict the crocodiles to sites that are supplied with fresh water, either constantly or for some part of the year. These high salinities can have direct effects on organisms, particularly in the early stages of their development. Continued monitoring of these variables, as well as the populations of crocodiles, should contribute to our understanding of the behavior of these populations of crocodiles prior to modification of their habitat, as well as determining the characteristics of the habitat they prefer. These are priorities for contributing to the improved management and conservation of this species.

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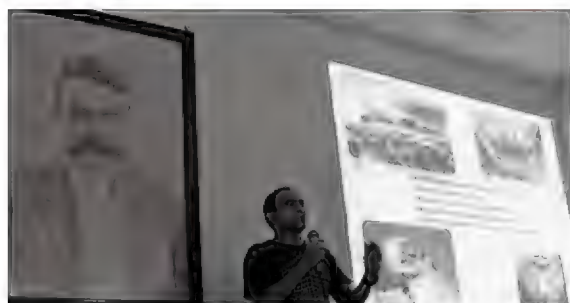
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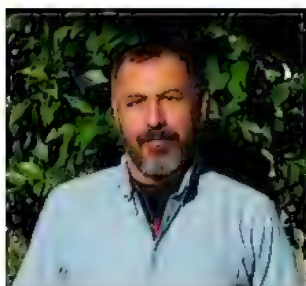
Sergio Aguilar-Olguín has a degree in Oceanography and a Master's degree in Marine Sciences, both from the School of Marine Sciences, Universidad de Colima (Mexico). Sergio is currently pursuing a Doctorate in Ecology and Management of Natural Resources at the University Center of the South Coast, Universidad de Guadalajara (Mexico), and is a professor for the honors degree in Biology in the Faculty of Biological and Agricultural Sciences, Universidad de Colima. Since 2001, Sergio has worked as head of the Department of Marine Turtles for the Management Unit, Conservation of Wildlife (UMA) Ecological Center of Cuyutlán "El Tortugario," where he coordinates activities on the protection and conservation of sea turtles and conducts studies on the population and reproductive ecology of sea turtles and crocodiles.



María Cruz Rivera Rodríguez is a graduate of the Faculty of Biological Sciences, Michoacán University of San Nicolás de Hidalgo, Mexico, with a Doctorate in Animal Science from the University of Colima, Mexico, with specializations in Ecology and Environmental Impact, as well as Environmental Education. María has served as Technical Manager of the UMA Ecological Center of Cuyutlán "El Tortugario," and a professor-researcher of the Faculty of Biological and Agricultural Sciences, University of Colima. She is a coordinator of research projects on wildlife management and conservation related to crocodiles, iguanas, sea turtles, snakes, lizards, and birds.



Helios Hernández-Hurtado is a Biologist who graduated from Universidad de Guadalajara, and received his Ph.D. from Universidad Autónoma de Nayarit, México. Helios has provided technical support to Reptilario Cipactli at Centro Universitario de la Costa, Universidad de Guadalajara (México) and is an active member of the Crocodile Specialist Group of IUCN. For 22 years, Helios has been working on the research, management, and conservation of crocodiles in captivity, as well as related projects on population ecology and management in the wilderness, in collaboration with local communities.



Ricardo González-Trujillo is a Biologist with a Ph.D. in Science in the area of Ecology and Natural Resource Management. Currently, Ricardo applies population genetics tools to solve problems related to ecology and the use and management of natural resources.



María Magdalena Ramírez-Martínez is currently a professor and researcher at Universidad de Guadalajara in Jalisco, México. Her research interests concern the ecology and biology of vertebrates, focusing on population dynamics and conservation, as well as the ecology of zoonotic diseases in western México. María enjoys traveling, long walks, and dance.



More road-killed Caspian Whipsnakes (*Dolichophis caspius*): an update on the species distribution along the Danube, in Romania

*Severus-D. Covaciu-Marcov, Alfred-Ș. Cicort-Lucaciu, Daniel-R. Pop,
Bogdan I. Lucaci, and Sára Ferenți

University of Oradea, Faculty of Informatics and Sciences, Department of Biology, Universității str. 1, 410087 Oradea, ROMANIA

Abstract.—*Dolichophis caspius* is present in Romania only in the southern regions, which partially represent its northern distribution limit. In recent years, new data on the distribution of *D. caspius* in the country have been collected, with many of these records being road-killed individuals. Conducted during 2013–2019, this survey further completes the information on this species' distribution in southern Romania, recording 55 (mostly new) distribution localities. *Dolichophis caspius* is a constant presence in the Danube meadow, and it also advances northwards along the Jiu, Olt, and Argeș River meadows. *Dolichophis caspius* was mostly found to inhabit areas with loess walls, but it was also present in flat areas with agricultural terrains. Most of these records were road-killed individuals. The high number of new distribution records could be driven by the last year's weather conditions, which were unusually warm and dry in the region. Also, the high number of road-killed snakes clearly reflects the increasing level of the human pressure on *D. caspius* in a region where natural habitats are becoming increasingly rare.

Keywords. Climate, Colubridae, habitat, loess walls, Reptilia, road mortality, Serpentes

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Introduction

The Caspian Whipsnake (*Dolichophis caspius*) is an Eastern-Mediterranean species (e.g., Tomović et al. 2014), present in Europe in its south-eastern regions, the Italian Peninsula, and the Balkan Peninsula (Sillero et al. 2014). A large part of its distribution range was probably populated shortly after the last glacial maximum (Nagy et al. 2010). The north-western limit of its distribution range reaches Hungary and Romania (see Nagy et al. 2010; Sahlean et al. 2014). In Hungary, it occupies only a very small territory near the Danube River, where it ascends farther north than in Romania, and in the last years new distribution records have been reported (e.g., Korsós et al. 2002; Bellaagh et al. 2008). Nevertheless, even the largest population in Hungary seems to be in decline (Frank et al. 2012). In Romania, *D. caspius* is present only in the southern part of the country, along the Danube River, especially in Dobruja (see Cogălniceanu et al. 2013; Sahlean et al. 2019). Its populations are considered to be declining in the country, with some regarded

as extinct (Iftime 2001–2002). The new distribution records of the last decades (Strugariu and Gherghel 2007; Covaciu-Marcov and David 2010; Sahlean et al. 2010; Ferenti et al. 2011; Covaciu-Marcov et al. 2012a) updated the knowledge on the species distribution in Romania (Cogălniceanu et al. 2013). However, *D. caspius* was still recorded in new localities in more recent years (Iftime and Iftime 2015, 2017; Sahlean et al. 2019), while large gaps in its known distribution range still remain (Sahlean et al. 2019). The majority of the recent distribution records represent road-killed individuals (e.g., Covaciu-Marcov and David 2010; Ferenti et al. 2011; Covaciu-Marcov et al. 2012a). Although these data indicate that *D. caspius* has a broader distribution range in Romania than was previously considered (Fuhn and Vancea 1961), they also confirm the pressure under which it is being subjected (Iftime 2001–2002). In the light of these facts, records of road-killed whipsnakes are of particular interest. Therefore, this note is an update on *D. caspius* distribution and road mortality intensity along the Danube in southern Romania.

Correspondence. *severcovaciu1@gmail.com

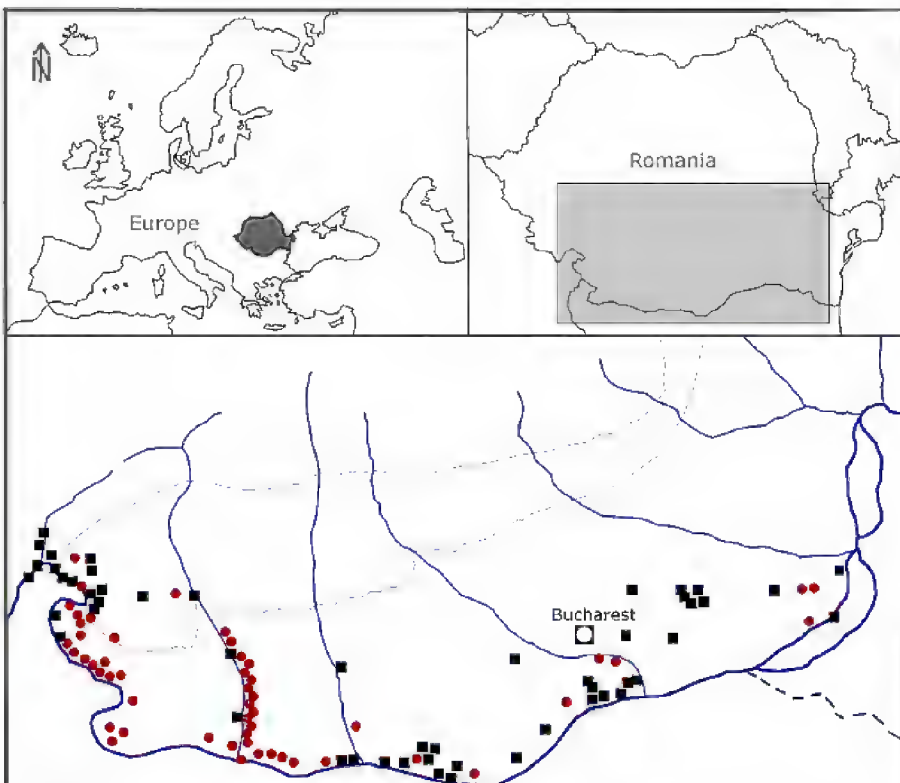


Fig. 1. *Dolichophis caspius* distribution in southern Romania. Squares: previous records (after Cogălniceanu et al. 2013; Iftime and Iftime 2016, 2017), red dots: new records.

Materials and Methods

The information on the distribution of *D. caspius* in southern Romania was obtained from field studies realized between 2013 and 2019. In 2016, 2017, and 2018, the Danube meadow between Drobeta-Turnu Severin and Fetești was surveyed once each year, in spring or in autumn. Additional surveys occurred 2–3 times each year in the western sector, from Drobeta-Turnu Severin to Bechet, and 1–2 times each year in the Jiu River meadow, between Craiova and Bechet. In total, ~29 days were spent in areas which could shelter *D. caspius* populations. None of those days were dedicated specifically to *D. caspius*, and snakes were observed by chance during other field work in the region (e.g., Covaciu-Marcov et al. 2017a,b, 2018). Road-killed individuals were observed directly from the car by driving at speeds of 50–60 km/h on roads with low traffic, a method previously used in the region (Covaciu-Marcov et al. 2012a). In some cases, direct searches for snakes were conducted in favorable habitats, and the observed individuals were recorded and photographed. Road-killed individuals were also photographed, and those in better physical condition were collected and deposited in the zoological collection of the University of Oradea, Romania.

Results

Dolichophis caspius individuals were recorded from 55 distribution localities in southern Romania (Table 1), most of which (52) were new distribution records (Fig. 1). The majority of the new distribution records are located in the western part of southern Romania, in the historical region of Oltenia, in Mehedinți and Dolj counties. Overall, 73 individual *D. caspius* snakes were identified. Five of the whipsnakes were encountered alive, while the others were found killed either by humans (two individuals), or by cars on the roads (Fig. 2). Juveniles and adults of up



Fig. 2. Road killed *Dolichophis caspius* individual from Lișteva, Dolj County, Romania.

to 1.60 m total length were both found killed on roads. *Dolichophis caspius* individuals were mostly found in spring (April–May) and autumn (September–October).

Discussion

Compared to the previously available data (e.g., Cogălniceanu et al. 2013; Iftime and Iftime 2015, 2017; Sahlean et al. 2019), the results reported here more than double the number of *D. caspius* records in the Danube meadow. This confirms once again that the species presents a continuous distribution in the region (e.g., Ferenți et al. 2011; Covaciu-Marcov et al. 2012a). While this fact was already well-established in the central part of the meadow (Sahlean et al. 2010; Ferenți et al. 2011; Cogălniceanu et al. 2013; Iftime and Iftime 2015, 2017), in the western parts the new data completed the *D. caspius* distribution range between Drobeta-Turnu Severin and the Olt River (Fig. 1), where a large gap has existed until now (Sahlean et al. 2019). These new records also show a continuous distribution along the Jiu River between Bechet and Craiova, and confirm the sporadic presence of this species north of Craiova (e.g., Sahlean et al. 2019). Although the three previously known distribution localities along the Jiu River already indicated this, Caspian Whipsnakes were rarely recorded in the region (e.g., Sahlean et al. 2019), despite the presence of apparently favorable habitats. The whipsnakes are also continuously distributed between Olt and Jiu Rivers. Based on only four records, *D. caspius* seems less common in the area between Calafat and Bechet. The records from the Argeș River region confirm that the species is currently well represented, although it was previously considered extinct from one locality (Iftime 2001–2002).

Table 1. *Dolichophis caspius* distribution records in southern Romania. Asterisks (*) indicate previous records. County abbreviations: Mh = Mehedinți County, Gr = Giurgiu County, Cl = Călărași County, Tr = Teleorman County, Ot = Olt County, Dj = Dolj County, Il = Ialomița County.

No.	County	Locality	Date(s)	Geographic coordinates	Road-killed	Human killed	Alive
1	Mh	Drobeta Turnu Severin - north	2 VI 2018 24 V 2015	44°39'28.90"N, 22°40'01.68"E	1 2	-	-
2	Mh	Gogoșu	3 VI 2018 6 X 2018	44°23'14.62"N, 22°33'26.21"E	3 3	-	-
3	Mh	Scăpău	14 X 2018	44°28'47.93"N, 22°43'34.29"E	1	-	-
4	Mh	Șimian	14 X 2018	44°35'53.62"N, 22°43'30.40"E	1	-	-
5	Mh	Chilia	21 VI 2016	44°29'02.32"N, 22°39'37.35"E	1	-	-
6	Mh	Izvoarele	21 VI 2016	44°17'48.87"N, 22°40'50.31"E	2	-	-
7	Mh	Pristol / Gârla Mare	21 VI 2016	44°13'08.71"N, 22°44'15.95"E	1	-	-
8	Mh	Vrata	21 VI 2016	44°12'13.75"N, 22°49'15.53"E	1	-	-
9	Mh	Batoți	15 X 2017	44°30'42.25"N, 22°40'22.16"E	1	-	-
10	Mh	Vânju Mare / Bucura	10 V 2014	44°24'39.12"N, 22°51'52.83"E	1	-	-
11	Mh	Arginești / Butoiești	28 VI 2015	44°34'39.33"N, 23°24'07.66"E	1	-	-
12	Mh	Devesel	X 2013	44°28'12.50"N, 22°41'07.16"E	1	-	-
13	Mh	Jiana Mare	IX 2014	44°24'20.97"N, 22°40'46.09"E	1	-	-
14	Mh	Gruia / Pătulele	V 2013	44°18'12.86"N, 22°44'27.07"E	1	-	-
15	Mh	Gruia	V 2013	44°17'00.83"N, 22°42'19.39"E	1	-	-
16	Mh	Balta Verde	30 IX 2019	44°20'17.70"N, 22°36'03.54"E	1	-	-
17	Mh	Izimșa	20 X 2019	44°10'48.17"N, 22°58'11.72"E	-	-	1
18	Gr	Hotarele	1 IX 2018	44°10'16.00"N, 26°22'10.25"E	1	-	-
19	Gr	Teiușu	1 IX 2018	44°10'06.40"N, 26°17'16.02"E	1	-	-
20	Gr	Plopșoru	1 IX 2018	44°00'39.49"N, 25°59'38.07"E	1	-	-
21	Gr / Cl	Greaca / Căscioarele*	12 IV 2014	44°07'37.37"N, 26°25'04.89"E	-	-	1
22	Tr	Zimnicele	1 IX 2018	43°39'52.82"N, 25°25'48.42"E	1	-	-
23	Tr	Seaca*	2 IX 2018	43°44'53.29"N, 25°04'07.76"E	1	-	-
24	Tr	Pleașov	23 IV 2017	43°51'13.39"N, 24°45'24.40"E	-	1	-
25	Tr	Năvodari	23 IV 2017	43°44'29.75"N, 25°06'48.68"E	1	-	-
26	Tr	Suhaia*	23 IV 2016	43°44'58.42"N, 25°13'40.21"E	1	-	-
27	Ot	Corabia E	2 IX 2018	43°46'13.59"N, 24°33'42.76"E	1	-	-
28	Ot	Potelu	12 IV 2014	43°45'21.57"N, 24°12'35.60"E	-	-	2
29	Ot	Ianca	IV 2013	43°46'45.28"N, 24°09'19.17"E	1	-	-
30	Dj	Călărași	2 IX 2018	43°47'11.23"N, 24°01'36.88"E	2	-	-
31	Dj	Bechet E	2 IX 2018	43°47'02.17"N, 23°58'43.39"E	1	-	-
32	Dj	Făcăi	13 X 2018	44°16'13.17"N, 23°49'16.72"E	-	1	-
33	Dj	Malu Mare	13 X 2018	44°15'23.80"N, 23°50'24.29"E	1	-	-
34	Dj	Malu Mare / Secui	13 X 2018, 14 X 2017	44°13'00.25"N, 23°51'49.08"E	1 1	-	-
35	Dj	Bratovoiești	13 X 2018	44°08'12.23"N, 23°53'50.21"E	1	-	-
36	Dj	Rojiște	13 X 2018, 4 X 2014	44°04'42.33"N, 23°55'58.69"E	2 1	-	-
37	Dj	Măceșu de Jos	13 X 2018 20 X 2019	43°52'49.20"N, 23°42'15.91"E	1 1	-	-
38	Dj	Maglavit	14 X 2018	44°01'29.78"N, 23°05'25.95"E	1	-	-
39	Dj	Tâmburești	14 X 2017	44°01'50.66"N, 23°56'04.04"E	2	-	-

Caspian Whipsnake distribution in Romania

Table 1 (continued). *Dolichophis caspius* distribution records in southern Romania. Asterisks (*) indicate previous records. County abbreviations: Mh = Mehedinți County, Gr = Giurgiu County, Cl = Călărași County, Tr = Teleorman County, Ot = Olt County, Dj = Dolj County, Il = Ialomița County.

No.	County	Locality	Date(s)	Geographic coordinates	Road-killed	Human killed	Alive
40	Dj	Murta	14 X 2017	43°58'42.86"N, 23°56'38.08"E	1	-	-
41	Dj	Toceni	14 X 2017	43°57'18.22"N, 23°56'40.57"E	1	-	-
42	Dj	Sadova / Căciulătești	14 X 2017	43°55'22.73"N, 23°57'09.75"E	1	-	-
43	Dj	Bechet to the harbour	14 X 2017	43°46'02.70"N, 23°57'30.91"E	1	-	-
44	Dj	Sadova	2 V 2014	43°54'37.18"N, 23°56'32.94"E	1	-	-
45	Dj	Lișteva	3 X 2015	43°50'32.23"N, 23°55'51.40"E	1	-	-
46	Dj	Dobroești / Toceni	14 X 2013	43°58'06.44"N, 23°56'39.17"E	1	-	-
47	Dj	Badoși	4 X 2014	44°08'29.93"N, 23°53'38.35"E	2	-	-
48	Dj	Rojiște S	4 X 2014	44°02'53.85"N, 23°56'10.46"E	1	-	-
49	Dj	Poiana Mare	5 X 2014	43°54'38.13"N, 23°03'51.85"E	2	-	-
50	Dj	Calafat	5 X 2014	43°58'45.62"N, 22°56'33.71"E	2	-	-
51	Dj	Zăval	30 VIII 2019	43°50'27.58"N, 23°50'57.37"E	1	-	-
52	Dj	Desa	29 IX 2019	43°52'47.14"N, 23°01'50.80"E	1	-	-
53	Il	Țândărei	28 VIII 2018	44°38'44.96"N, 27°41'06.00"E	1	-	-
54	Il	Țândărei / Mihail Kogălniceanu	25 VIII 2019	44°39'11.49"N, 27°42'29.19"E	1	-	-
55	Il	Buliga West	30 VIII 2019	44°21'09.69"N, 27°47'07.46"E	-	-	1

The new records confirm the association of *D. caspius* with the loess walls of the Danube meadow (e.g., Ferenți et al. 2011; Covaciu-Marcov et al. 2012a). Most observations were made in areas with loess walls, both along the Danube meadow and its main tributaries (Jiu, Olt, Argeș). Along the Danube, loess walls have southern exposure; while along the Jiu (Fig. 3) and Olt Rivers they have western exposure, with southern oriented meadows, and along the Argeș River they are exposed to the north. Only some localities, such as Măceșu de Jos, are devoid of loess walls, with a plain relief and an indistinguishable boundary between the meadow and its neighboring terraces. In such cases, agricultural lands surrounding the roads were used by *D. caspius*, as is found in other parts of its distribution area (e.g., Ioannidis and Bousbouras 1997; Arslan et al. 2018; Sahin and Afsar 2018). Thus, the loess walls are confirmed to be favorable habitats for *D. caspius* (e.g., Korsós et al. 2002; Bellaagh et al. 2008), but some individuals persist even in flat habitats.

The data presented here extend the northern limit of the distribution of *D. caspius* along the Danube tributaries. Whipsnakes are xerophilous, influenced by temperature and precipitation, and so the future northward distribution could be facilitated by climate change (see Sahlean et al. 2014), and even hatching time depends regionally on temperature (Sahlean and Strugariu 2018). In south-western Romania, the last few decades were warmer and drier than longer term averages (Pravalie et al. 2014), and *D. caspius* was mostly recorded in the regions of the country with a drier climate (Matei et al. 2016). The high

number of whipsnakes found could be a consequence of favorable climatic conditions that enabled an increase in the population growth rate. Moreover, *D. caspius* could already be at an advantage due to climate change, despite the high number of road-killed individuals. Nevertheless, until recently the Danube meadow was mostly covered by wet areas, lakes, or forests (probably except for the loess walls), which are now drained, deforested, and replaced with agricultural terrains (e.g., Licurici 2011; Benecke et al. 2013; Geacu et al. 2018). Thus, agriculture probably enlarged *D. caspius* habitats beyond the loess walls. This scenario is supported by previous records of this species in the agricultural areas of other regions as well (e.g., Ioannidis and Bousbouras 1997; Arslan et al. 2018; Sahin and Afsar 2018).

The high number of *D. caspius* records is not necessarily an indicator of favorable conditions, but



Fig. 3. *Dolichophis caspius* habitat in Jiu meadow, Romania.

clearly suggests a great human pressure in which snakes are disturbed by intensive agriculture and habitat alteration. In trying to avoid this impact, they move more often, more frequently falling victim to the increasing road traffic. Increased traffic intensity is known to increase road mortality (e.g., Jones et al. 2014; Miranda et al. 2017; Gonçalves et al. 2018), even if *D. caspius* is the fastest snake in Romania (Fuhn and Vancea 1961). In addition, the greater activity of herpetologists may have increased the number of records and observed road-killed individuals. Nevertheless, we have no prior information on the species status before the extensive alteration of the Danube meadow (e.g., Licurici 2011; Benecke et al. 2013; Geacu et al. 2018). *Dolichophis caspius* records based on road-killed individuals are not specific only to Romania (Covaciu-Marcov and David 2010; Ferenti et al. 2011; Covaciu-Marcov et al. 2012a; Sahlean et al. 2019), but are also commonly reported in other regions (e.g., Korsós et al. 2002; Krčmar et al. 2007; Kambourova-Ivanova et al. 2012; Mollov et al. 2013). Most of the whipsnakes found were killed on roads situated at the upper limit of the loess walls, just as previously indicated (Covaciu-Marcov et al. 2012a). Road-killed individuals were rarely recorded at the base of the loess walls, or in regions without loess walls.

In southern Romania, *D. caspius* has arrived only recently, at the end of its postglacial expansion (Nagy et al. 2010). It uses natural and restrictive, but also modified, habitats. Its protected status (O.U.G 57/2007, Monitorului Oficial al României, <http://www.monitoruloficial.ro/>) increases the importance of the regional biodiversity, where numerous relicts are present (e.g., Paşcovschi 1967; Ferenti and Covaciu-Marcov 2014; Covaciu-Marcov et al. 2017a, 2018). *Dolichophis caspius* is not the only southern species with its northern distribution limit in this region, but there are also other amphibian and reptile species in the same category (e.g., Covaciu-Marcov et al. 2012b; Cogălniceanu et al. 2013; Székely et al. 2013).

The records of *D. caspius*, especially road-killed individuals, have been increasing in recent years in Romania (e.g., Strugariu and Gherghel 2007; Covaciu-Marcov and David 2010; Sahlean et al. 2010; Ferenti et al. 2011; Covaciu-Marcov et al. 2012a; Iftime and Iftime 2015, 2017; Sahlean et al. 2019). The finding of numerous snakes is a consequence of what kills them, namely the road network development. The impact of roads on biodiversity is increasingly clear, including in Romania (e.g., Cicort-Lucaciu et al. 2012, 2016; Ciolan et al. 2017; Covaciu-Marcov et al. 2017c; Popovici and Ile 2018; Teodor et al. 2019). The proposal of speed reductions in regions populated by whipsnakes (Covaciu-Marcov et al. 2012a) is no longer feasible because, according to the new distribution records, the speed limits would need to be applied to hundreds of km of the roadway. If the protection of relicts strictly related to natural habitats is relatively facile, depending on the

conservation of the last remnants of initial habitats, then the conservation of a species (such as *D. caspius*) which recently arrived at its postglacial expansion (Nagy et al. 2010) and is probably influenced by the climatic changes (Sahlean et al. 2014) or even by some human impacts, is much more difficult. This difficulty is further compounded by the fact that this species could enter in conflict with the exact factors that favor it at the moment.

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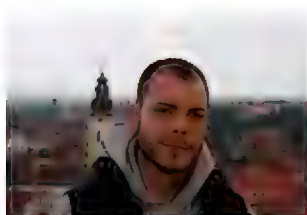
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Severus-Daniel Covaciu-Marcov, Ph.D., is a Romanian biologist and author of faunistic studies on herpetofauna, as well as other animal groups, from Romania. Many of his studies were realized in protected areas. Sever is mainly interested in the biogeography of different animal groups, but also in the anthropic impacts upon the fauna. In this regard, he has published studies on road mortality, invasive species, and other issues. Sever is an Associate Professor at the University of Oradea, Faculty of Informatics and Sciences, Department of Biology, in Oradea, Romania.



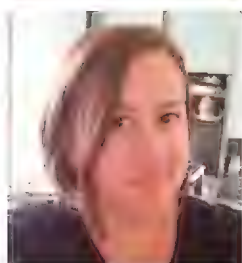
Alfred-Ştefan Cicort-Lucaciu, Ph.D., is a lecturer at the University of Oradea, Faculty of Informatics and Sciences, Department of Biology. His research is mainly focused on the biodiversity of wetlands, while most of his publications concern herpetology. Alfred's Ph.D. thesis focused on the biology, ecology, and distribution of salamanders and newts in Romania.



Daniel-Răzvan Pop is an associate assistant at the University of Oradea, Faculty of Informatics and Sciences, Department of Biology, and a 1st year Ph.D. student in the Doctoral School of Biomedical Sciences, Domain Biology of the same university. Daniel's studies are now focusing on railway ecology, but he has also worked on studies concerning fauna distribution and anthropogenic impacts.



Bogdan-Ionuţ Lucaci is a 3rd year B.Sc. student at the University of Oradea, Faculty of Informatics and Sciences, Department of Biology. Bogdan is interested in wildlife ecology and biogeography, focusing especially on certain groups of vertebrates, such as amphibians and reptiles. He is an enthusiastic participant in different studies within the domains of biodiversity conservation in natural protected areas and human impacts upon the fauna.



Sára Ferenţi, Ph.D., is a biologist from Romania, with a special interest in the geographic distribution and ecology of terrestrial isopods and herpetofauna. She has conducted studies on the fauna of some natural protected areas and some endemic species from Romania, but also on the anthropogenic impacts upon the fauna. Sára is an Assistant Professor at the University of Oradea, Faculty of Informatics and Sciences, Department of Biology.



Feminization tendency of Hawksbill Turtles (*Eretmochelys imbricata*) in the western Yucatán Peninsula, Mexico

^{1,2,*}Cynthia Dinorah Flores-Aguirre, ³Verónica Díaz-Hernández, ⁴Isaías Hazarmabeth Salgado Ugarte, ²Luis Enrique Sosa Caballero, and ^{2,*}Fausto R. Méndez de la Cruz

¹Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, A.P. 70-153. C.P. 04510, Ciudad de México, MÉXICO ²Instituto de Biología, Departamento de Zoología, Universidad Nacional Autónoma de México, Circuito exterior sin número, Ciudad Universitaria, Copilco, Coyoacán, cp 04510, Ciudad de México, MÉXICO ³Facultad de Medicina, Departamento de Embriología, Universidad Nacional Autónoma de México, Circuito Interior Ciudad Universitaria, Avenida Universidad n. 3000, cp 04510, Ciudad de México, MÉXICO ⁴Facultad de Estudios Superiores Zaragoza, Campus dos, Batalla 5 de mayo s/n esquina Fuerte de Loreto, Colonia Ejército de Oriente, Iztapalapa c.p. 09230, Ciudad de México, MÉXICO

Abstract.—The viability of sea turtle populations during their early stages depends mostly on hatching success and a balanced sex ratio of the hatchlings. In sea turtles, sex is determined by the temperature at which the eggs are incubated. Consequently, climate change can play a critical role in population fitness, because increased temperatures can skew the sex ratio towards females. The Hawksbill Turtle is a Critically Endangered species and Mexico holds one of the largest populations of nesting females worldwide. Given the importance of Mexican Hawksbill populations, it is necessary to evaluate their nest temperatures, particularly during the thermosensitive period, and identify the sex ratio of the hatchlings. Hawksbill Turtle nests were characterized in three nesting beaches from the western Yucatán Peninsula, Mexico, to determine the temperatures of the nests *in situ* and the sex ratio of the hatchlings. The results showed that the incubation temperature was warmer than the pivotal temperature by 1.255 ± 0.18 (SE) °C, and in 82.05% of the nests monitored with only female hatchlings. This was also confirmed via histology of the gonads of dead hatchlings. The amount of shade above the nests and rainfall were the most influential factors for decreasing nest temperatures. This study shows a trend toward the feminization of hatchlings due to the low percentage of shade above nests which is able to increase the sex ratio bias due to coastal dune deforestation, together with the increase in environmental temperatures due to climate change. The skewed ratio toward females could have negative consequences for the maintenance of sea turtle populations on the Yucatán Peninsula, so is necessary to develop strategies of conservation in order to reduce this trend.

Keywords. Anthropogenic impact, climate change, coastal-dune vegetation, female hatchlings, gonad histology, nest temperature, pivotal temperature, rain, shade, temperature-dependent sex determination

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Introduction

Temperature-dependent sex determination occurs in a variety of reptiles, including all sea turtles (Bull and Vogt 1979; Wibbels 2003). In these species, the temperature that embryos experience during their development determines the sex of hatchlings. Specifically, sex determination occurs in the thermosensitive period (TSP), which is a particular time during embryonic development (the middle third of incubation) when the temperature affects gonad differentiation (Ciofi and Swingland 1997; Mrosovsky and Pieau 1991). The TSP provides a balance between both sexes at a certain temperature, called the

pivotal temperature (PT), and increasing or decreasing the temperature relative to the PT shifts the sexual proportion towards one sex (Mrosovsky and Yntema 1980). In sea turtles, the sex ratio is skewed toward males at temperatures below the PT and toward females at temperatures above the PT (Salame-Méndez 1998). Since the PT for all sea turtles is between 28 °C and 30 °C (Hawkes et al. 2009), the increase in temperature due to global warming of the planet could skew the sex ratio.

A bias towards feminization has been reported in all species of sea turtles in multiple studies around the world since 1997 (Hawkes et al. 2009), being less represented in Hawksbill Turtles (*Eretmochelys imbricata*; Hawkes

Correspondence. *dcynthia.fa@gmail.com (CDFA); roveazdih@yahoo.com.mx (VDH); isalgado@unam.mx (IHSU); qiqesc@gmail.com (LESC); *faustor@ib.unam.mx (FRMC)

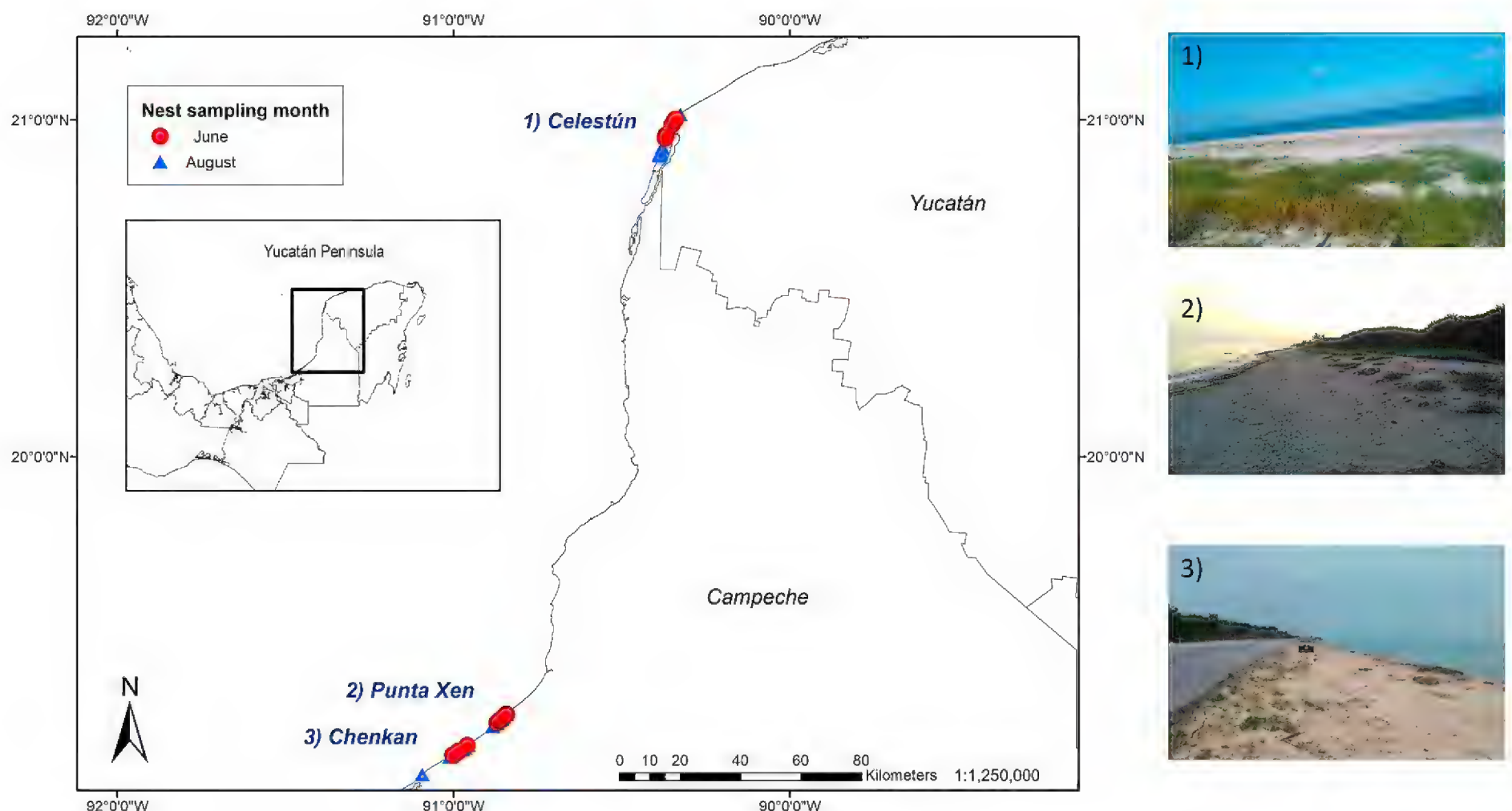


Fig. 1. Geographical locations of the monitored nests of *Eretmochelys imbricata*, as well as the beaches studied and their images. 1) Celestún, 2) Punta Xen, and 3) Chenkan.

et al. 2009; Hays et al. 2017); even though Hawksbills are considered Critically Endangered (see <https://www.iucnredlist.org/search?taxonomies=100888&searchType=species>). Thus far, no studies have addressed incubation temperatures on Mexico's nesting beaches, even though the Yucatán Peninsula hosts one of the largest populations of Hawksbill females worldwide and the largest nesting population of Hawksbill Turtles in the Atlantic (Campbell 2014; Garduño-Andrade and Guzmán 1999; Meylan and Donnelly 1999; Mortimer and Donnelly 2008).

Sea turtles select microenvironments for nesting, thereby manipulating the thermal conditions to which their eggs will be exposed during incubation (Diffenbaugh and Field 2013). Thus, the nest temperature will be modified according to its location with respect to several factors, including vegetation (Kamel 2013; Kamel and Mrosovsky 2006), distance to the coastline (Rees and Margaritoulis 2004), sand differences in albedo and grain size (Hays et al. 2001; Schaetzel and Anderson 2005), and others. In addition, the thermal properties of the beaches can provide different incubation temperatures (Hawkes et al. 2009), and the temperature varies across the nesting season (Mrosovsky et al. 1984).

Therefore, this study was carried out on three beaches in the western Yucatán Peninsula with different degrees of anthropogenic impact (disturbance due to human infrastructures like roads, building for tourists, vegetation removal, etc.) as well as variations in the littoral characteristics (width of the beaches, distribution and type of natural vegetation). These differences are expected to promote diverse thermal gradients which modify the nest temperatures, and, consequently, sex ratios and hatching success. It is known that the least

preserved beaches (especially in the dune strip) are those that maintain the highest average temperatures and decrease the hatching success (Pike 2008, 2009), particularly for Hawksbill Turtles whose nesting is closely related to vegetation (Horrocks and Scott 1991; Kamel 2013). Therefore, the objectives of this study were to determine the temperatures of Hawksbill Turtle nests *in situ* during the TSP and estimate their sex ratios based on a histological calibration, as well as determine if there are differences in the sex ratio over the course of the nesting season and among beaches.

Materials and Methods

Fieldwork was carried out on three index nesting beaches (historical localities with higher nesting females) of the Hawksbill Turtle in the western Yucatán Peninsula, Mexico. The beaches were: Chenkan (19°07'50"N; 90°31'13.15"W) and Punta Xen (19°12'42.57"N; 90°52'07.01"W) in the state of Campeche, and Celestún (20°59'33.72"N; 90°24'54"W) in the state of Yucatán (Fig. 1). These beaches were selected as they present differences in their littoral characteristics (width of the beach and type of vegetation) and in their conservation status (degree of disturbance due to human infrastructure). Celestún is the most well-preserved beach (with wider beaches, low disturbance in vegetation, limited erosion and few human infrastructures on most of the beach), while Chenkan is the least preserved (with high erosion and deforestation rates, and a road along the beach). The study was conducted in 2017, during the middle (June–July) and end (August–September) of the nesting season. June is the month when the nesting increases

(Guzmán et al. 2008), so it would be more representative to sample then, while in August the temperature begins to decrease due to the increase in rainfall (García 2004). These differences could allow the evaluation of distinct responses to the temperature during nesting, which would modify the sex of the hatchlings and would therefore provide greater variability in the samples.

The permit to handle the Hawksbill Turtles was provided by SEMARNAT (SGPA/DGVS/03995/17). The fieldwork consisted of making routes from 2100 to 0500 h, searching for nesting Hawksbill Turtles randomly but trying to include different thermal zones (i.e., under high vegetation (HV), under low vegetation (LV), and without vegetation (WV), at different distances along each beach). The study was conducted using only nests *in situ* (i.e., places that females naturally selected to lay their eggs), so once the turtles began to oviposit, the nest location data were taken with a Global Positioning System (GPS), as the nests were left exactly where the turtles nested.

The characterization of each nest involved the following procedure. The temperatures (°C), both inside and outside the incubation chamber, were taken with a digital thermometer (Fluke, 51-II®). The depth of the nest from the first to the last egg deposited was measured with a flexometer. Temperature data loggers (HOBO® pendant) were placed in the middle of the nests and temperature data were recorded every half-hour throughout the incubation period. The distances from the nest to the nearest vegetation and to the coastline were measured with a flexometer. The type of vegetation closest to the nest was recorded, and the percentage of shade above the nest was established when checking the nest close to noon.

The hatching success was determined according to Miller (1999), by checking the nests once the hatchlings had left, after 55 ± 5 d incubation (Guzmán 2008). At the hatchling stage, sea turtles cannot be sexed by external morphology (Merchant 1999; Wibbels 2003). Therefore, the sex ratios of the Hawksbill hatchlings were estimated using the temperatures recorded by the data logger during the TSP, which were analyzed by the TSD software version 4.0.3 designed by Girondot (1999). In addition, the temperature used to determine the sex in the software was calibrated based on the histology of the dead hatchlings, as this option avoided destructive sampling of this Critically Endangered species. Any dead hatchlings found in the monitored nests were collected to dissect the urogenital complex (mesonephros and gonads), which was fixed in 4% paraformaldehyde and transported to the Laboratorio de Embriología de la Facultad de Medicina, Universidad Nacional Autónoma de México. In the laboratory, the samples were dehydrated by gradual ethanol solutions and maintained in 75% ethanol. Later, they were embedded in histological paraffin and the gonads were sectioned (between 8 and 12 μ m thick) using a rotary microtome. Subsequently,

the samples were stained with periodic acid-Schiff's reaction and mounted on slides for observation under an optical microscope. Under the microscope, the ovaries or testes were identified using the criteria of Yntema and Mrosovsky (1980) and Wibbels et al. (1999).

Sex ratio results obtained from gonad histology were used as input for the TSD software to obtain a field estimate of the PT for this species. Subsequently, this PT was used as a formula parameter for estimating the hatchling's sex ratios, based on the mean incubation temperature during TSP recorded in each nest. Since the TSD software was developed for use with data derived from constant temperature incubations, the average temperature (and standard deviation) of each nest was measured during each third of the incubation period, in order to gauge the temperature variability within the nests.

Since the shade provided by the HV decreases the temperature of the nests (Kamel 2013; Kamel and Mrosovsky 2006), the percentages of nests that would have temperatures close to the PT (shaded nests) and nests that would have feminizing temperatures (sun-exposed nests and those under LV) were estimated according to the thermal zones (HV, LV, and WV) along each beach. The thermal zones of the beaches were categorized based on the width (distance from the coastline to the HV or limit where turtles could not nest, such as a human infrastructure) and the dominant vegetation every 2 km on Chenkan (18 km) and Punta Xen (18 km) beaches, and every 5 km on Celestún (22 km). These measurements of the beaches, together with the geographical locations recorded for the nests, were used to correlate them with satellite images and the maximum distance of the nests from the coastline (according to our results), through Google images, in each beach per km (in linear m).

To document the degree of anthropogenic impact on each beach, Google satellite images were used to measure (in linear m) the disturbance along each beach, and thereby estimate the percentage of human infrastructures that would affect the nesting of the turtles per km. To establish the number of nests per km throughout the entire nesting period of the Chenkan and Punta Xen beaches, databases were obtained from the Comisión Nacional de Áreas Naturales Protegidas (CONANP) and Grupo Ecologista Quelonios A.C., respectively. The precipitation levels on the three beaches during the entire nesting period were obtained from the Comisión Nacional del Agua (CONAGUA), in order to relate the dates of both incubation periods with the amount of rain.

Statistical Analysis

The normality and homogeneity of the data were tested by Kolmogorov-Smirnov and Levene's tests. Wilcoxon tests (as the distribution was not normal) were used to compare the nest temperatures, hatching success, and the variables that characterize the nests (incubation

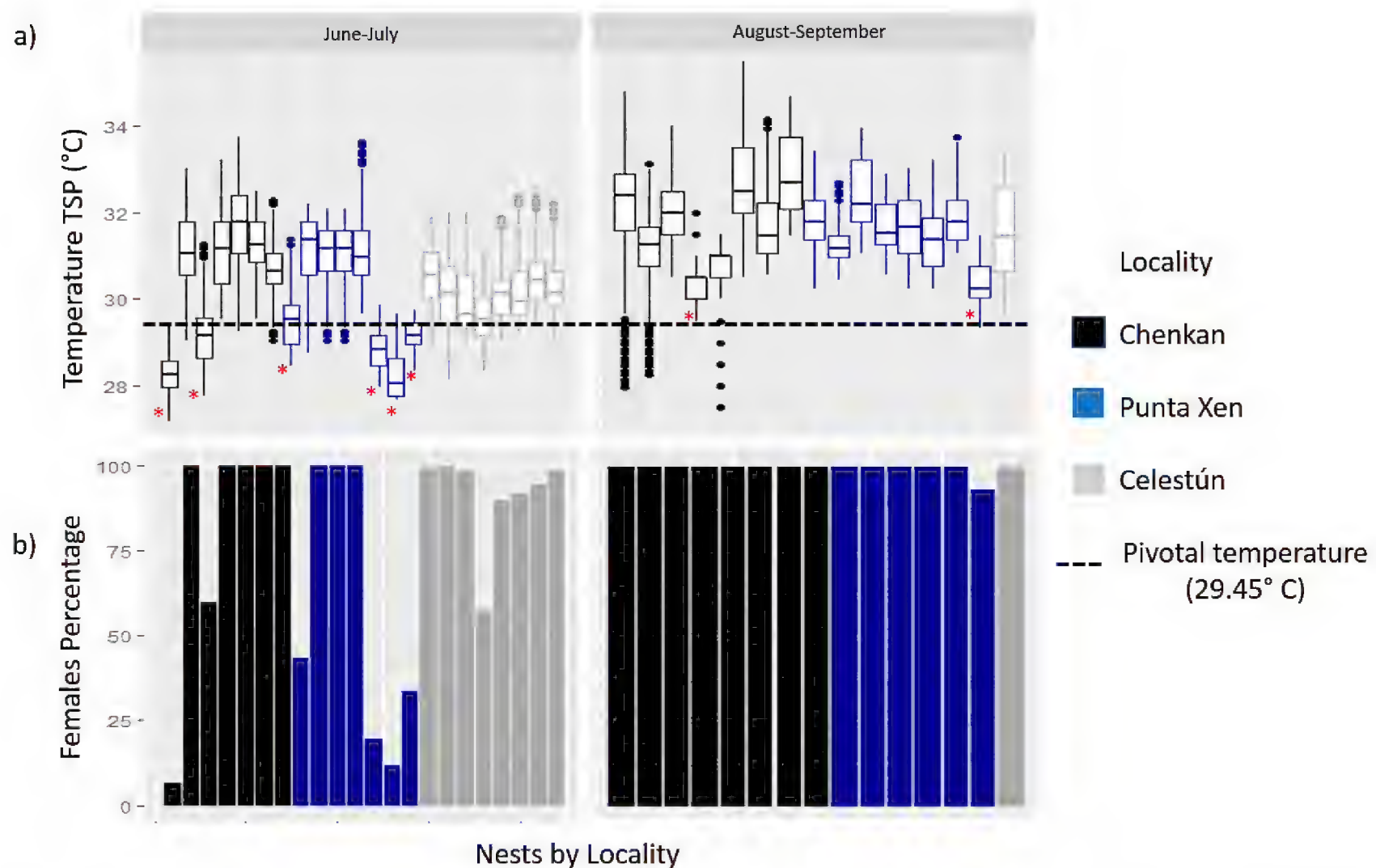


Fig. 2. (a) Incubation temperatures of *Eretmochelys imbricata*, during the TSP, on the beaches of Chenkan, Punta Xen, and Celestún, during two incubation periods (June–July and August–September). The dotted line indicates the pivotal temperature. **(b)** Percentages of females in the monitored nests on the nesting beaches of the western Yucatán Peninsula (Mexico). The red asterisks show where the differences were significant ($p < 0.05$) among nests, by beach.

chamber temperature, depth, distance to the vegetation and to the coastline, as well as percentage of shade above the nests) between the two incubation periods (June–July and August–September) within each beach. An ANOVA was performed to compare the different variables that characterize the nests among the three monitored beaches. To determine the TSP of each nest, the days from the beginning to the end of incubation were calculated, and the middle third of the incubation period was established. Subsequently, the temperature during the TSP was compared among the beaches by a Generalized Linear Model (GLM), with the set of nest temperatures at each beach as the response variable. To compare the temperatures during the TSP among the nests on each beach, a GLM was used, with the temperatures recorded for each nest as the response variable. In addition, a time series was used, where nest temperatures were correlated with the time at which they were taken, and were compared among all the nests for each locality. Measuring these correlations established the time required for shaded nests to absorb heat, in comparison to sun-exposed nests. Statistical significance was set at $p < 0.05$; and SPSS, Stata SE11, and R version 3.5.1 (R Core Team 2018) were used for statistical analyses.

Results

During the 2017 nesting season, 20 nests were characterized on Chenkan beach (10 in June and 10 in August), 20 on Punta Xen beach (10 in June and 10 in

August), and 12 on Celestún beach (eight in June and four in August). The nests characterized along the three monitored beaches showed no significant differences in the temperature of the incubation chamber, distance to the coastline, or percentage of shade ($F_2 = 1.614$, $p = 0.214$). However, the distance to the nearest vegetation differed significantly among beaches, with nests in Celestún being the farthest from vegetation ($F_2 = -3.514$, $p = 0.042$). Likewise, nest depth differed significantly among the beaches, with the nests on Chenkan beach being the most shallow ($F_2 = 3.85$, $p = 0.036$; Table 1).

The average temperatures of the monitored nests did not vary significantly among the three beaches during June–July ($F_2 = 0.202$, $p = 0.819$), nor in August–September ($F_2 = 0.462$, $p = 0.640$, Table 2). The average incubation temperature on Chenkan beach was higher in August–September (31.89 ± 1.36 °C) than in June–July (30.78 ± 1.08 °C, $F_{11,73} = 2.246$, $p = 0.045$). In Punta Xen, only the average temperature during the TSP was higher in August–September (31.386 ± 1.164 °C) than in June–July (29.869 ± 0.728 °C, $F_{10,18} = 2.857$, $p = 0.017$; Table 2). The incubation temperatures across all beaches and the entire nesting season were within the thermal interval that allows embryonic development (28.5 to 32.9 °C, Table 2, Fig. 2). The nest temperatures recorded during the TSP of June–July at Chenkan beach showed significant differences among them, according to their locations ($F_7 = 34.830$, $p < 0.001$), and the same was found at Punta Xen beach ($F_7 = 49.911$, $p < 0.001$). For the nests that were found under the shade, more than

Table 1. Nest characteristics of Hawksbill Turtles in the western Yucatán Peninsula, Mexico.

Locality	Nesting (month)	Internal temperature (°C)	External temperature (°C)	Total depth (cm)	Depth of the last egg (cm)	Distance to vegetation (m)	Distance to coastline (m)	Percentage of shade (%)
Chenkan	June	29.73 ± 1.49	26.8 ± 1.29	32.1 ± 6.85	14.73 ± 7.16	1.15 ± 1.86	17.79 ± 5.09	36.67 ± 46.90
	August	29.38 ± 1.94	26.62 ± 2.54	44.75 ± 7.18	25.41 ± 8.51	0.49 ± 1.48	15.06 ± 2.43	9.57 ± 19.12
Punta Xen	June	29.19 ± 0.59	27.40 ± 0.86	34.58 ± 6.72	21.13 ± 3.98	0.86 ± 1.50	13.04 ± 10.07	46.11 ± 15.95
	August	28.3 ± 1.70	27.57 ± 1.15	46.45 ± 7.56	28.65 ± 7.81	1.78 ± 3.52	19.78 ± 6.53	24.44 ± 34.68
Celestún	June	28.62 ± 0.55	26.84 ± 1.12	37.57 ± 6.03	20.93 ± 4.44	4.07 ± 4.68	19.74 ± 6.09	26.67 ± 15.28
	August	28.78 ± 0.46	26.98 ± 1.27	49.67 ± 4.62	35.33 ± 7.51	2.45 ± 0.97	20.48 ± 5.53	22.5 ± 10.61

70% of their total coverage throughout the incubation period had significantly lower temperatures compared to sun-exposed nests ($p < 0.001$, Fig. 2a). On Celestún beach, the nest temperatures did not show significant differences between these two conditions of shaded (with less than 50% of total coverage) and sun-exposed. The nest temperatures in the TSP of August–September at Chenkan beach showed significant differences only in one nest, with lower temperatures compared to the other nests ($F_7 = 11.320$, $p < 0.001$), and the same pattern occurred on Punta Xen beach ($F_7 = 13.493$, $p < 0.001$). Both of these nests on each beach were sun-exposed, therefore they remained above the PT (Fig. 2a). The nests that were found under total shade coverage throughout the incubation period, exhibited a temperature damping effect, taking 3.5–4 h longer to heat up to the same temperature as the sun-exposed nests ($p < 0.001$). Although the shaded nests had lower temperatures than those that were sun-exposed, most of the nests were exposed; and therefore, in general, the nest temperature during the TSP was hotter than the PT.

The hatching success for June–July did not differ significantly between the three beaches: Chenkan ($61.9 \pm 26.71\%$), Punta Xen ($57.97 \pm 41.04\%$), and Celestún ($71.38 \pm 24.53\%$, $F_2 = 0.54$, $p = 0.589$). A similar trend was observed in the period of August–September on Chenkan ($50.89 \pm 24.36\%$), Punta Xen ($26.02 \pm 36.3\%$), and Celestún ($61.95 \pm 41.94\%$, $F_2 = 2.30$, $p = 0.125$), although numerically lower hatching success was observed in the nests located in Punta Xen (Fig. 3).

During the cleaning of the nests in both nesting periods, 170 dead hatchlings were collected from 17 nests (five from Chenkan, five from Punta Xen, and seven from Celestún). In a nest from Chenkan beach (June–July), a sample of 31 dead hatchlings was obtained, which were predated by ghost crabs. In addition, on Punta Xen beach

(August–September), a sample of 65 dead hatchlings was obtained, which were predated by ants (*Solenopsis germinata* and *Labidus coecus*). From these two nests, the largest and freshest samples were obtained because the hatchlings were found less than 12 h after death. In five nests, the hatchlings required more than 36 h to emerge from the nests, therefore the tissues of dead hatchlings were degraded and the sexes of 25 samples were unidentifiable. In total, 145 gonads from 12 nests were identified. Histological examination showed that the ovaries of hatchlings exhibited a thickened cortex and unorganized medulla, whereas testes presented a very thin cortex and had an organized medullary region with developing seminiferous cords (Fig. 4). From the total sample, 126 females and 19 males were determined. Only hatchling females were found in the sun-exposed nests, with temperatures of 30.21 °C and above. However, both sexes were found in the shaded nests, with temperatures in the range of 28.55–29.59 °C (Table 3). The temperature variation in the nests during the TSP, from which the histological samples were obtained, was 0.77 ± 0.15 °C. These results were used as the input and for calibration of the TSD software, which determined the pivotal temperature as $29.45 (\pm 0.147 \text{ SE})$ °C, according to the maximum-likelihood analysis, with the Richard model ($p_{(10)} < 0.001$, AIC = 61.54, Fig. 5). In addition, the transitional range of the temperature was -0.53 ± 0.048 . According to the TSD software, only 27.02% of the hatchlings were males in the monitored nests. The highest percentage of females occurred during the late nesting season (August–September, $83.33 \pm 4.01\%$) and the lowest percentage occurred during the middle (June–July, $65.49 \pm 4.08\%$, $t_{222} = -3.72$, $p = 0.006$; Fig. 2b).

In the three studied beaches, the dominant vegetation was the coastal dune halophyte, which is mainly low and medium-sized, characterized by herbaceous plants

Table 2. Average nest temperatures during the total incubation period and the TSP of the Hawksbill Turtles for each monitored beach, between the months indicated. Significant differences ($p < 0.05$) are indicated by asterisks.

Beach	Temperature during incubation (°C)		Temperature during TSP (°C)	
	June–July	August–September	June–July	August–September
Chenkan	30.78 ± 1.08*	31.9 ± 1.36*	30.49 ± 0.72*	31.71 ± 0.89*
Punta Xen	30.44 ± 1.44	31.36 ± 1.23	29.87 ± 0.62*	31.63 ± 0.68*
Celestún	30.85 ± 1.48	30.4 ± 0.95	30.29 ± 0.83	30.1 ± 0.63

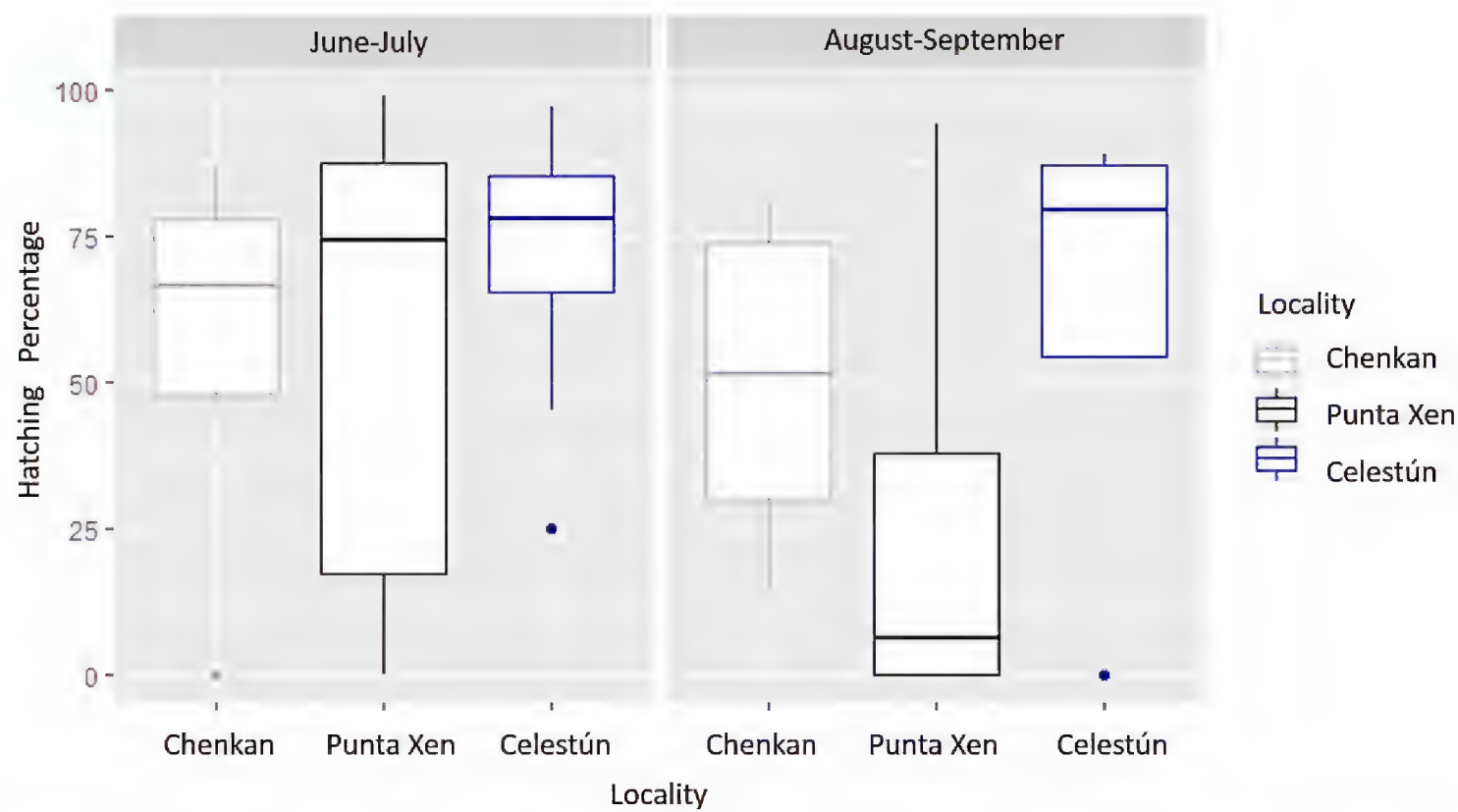


Fig. 3. Hatching success of *Eretmochelys imbricata* on the beaches of Chenkan, Punta Xen, and Celestún, Yucatán Peninsula (Mexico).

and shrubs. However, the high vegetation (HV; i.e., the vegetation that provides more shade) changed among the three beaches. In Chenkan, the HV was dominated by spots of coconut plantations, present only in 22.2% of the total length of the beach, because natural vegetation (mangroves) was located on the other side of a road. In Punta Xen, the dominant HV was the mangroves, present in 62.5% of the total length of the beach; whereas in Celestún, the HV was dominated by tropical dry forest, which corresponded to 77.3% of the total length of the beach. Nevertheless, the percentage of shade provided by the HV to the nests was not equal to the percentage of HV along the three beaches studied, since it depended on the distance between the HV, the nests, and the coastline, as well as the degree of anthropogenic disturbance. Thus, according to the distance from the coastline to the HV and the maximum distance between the monitored nests and the coastline (30 m; Table 1) the percentage of the shaded nest was calculated. Chenkan beach had the lowest percentage of shaded nests, with only 2.89%, next was the Celestún beach with 21.54%, and the highest

was the Punta Xen beach with 29.59%. The percentage of shade above the monitored nests on the three beaches also changed between the periods of June–July and August–September. In June–July, 50% of the nests on Celestún were recorded under shrubs, 50% of the nests in Punta Xen were under the shade of the mangroves, and 40% of the nests in Chenkan were under shade (coconuts and man-made structures to create shade on the beach, known as *palapas*). In contrast, in August–September, most of the nests were found in less shaded areas: in Celestún, 0% of the monitored nests were under shade, in Punta Xen, 20% nests were shaded, and in Chenkan, 10% of nests were shaded.

According to the analysis of anthropogenic impacts, Chenkan beach exhibited a 100% disturbance, mainly due to the road along the beach. On average, the distance was 17.38 ± 7.55 m from the coastline to the road in the most eroded area, which caused the nesting of the turtles to be interrupted. In contrast, the wider area from the road to the coastline had an amplitude of 140.57 m, however, it had high deforestation and human infrastructures, such

Table 3. Numbers of dead hatchlings and percentages of females, according to the histological sex, temperature during the TSP at which nests were incubated *in situ*, and the percentage of shade above the nests, by locality and incubation period.

Period	Beach	Shade (%)	No. dead hatchlings	Temperature during TSP (°C)	Females (%)
June–July	Chenkan	100	3	29.59 ± 0.52	67
		0	31	30.66 ± 0.44	100
	Punta Xen	100	6	28.55 ± 0.66	17
		90	8	28.84 ± 0.51	25
		70	3	29.35 ± 0.59	33
	Celestún	60	10	29.6 ± 0.64	50
		20	2	30.21 ± 0.67	100
		40	3	30.44 ± 0.82	100
		40	4	30.93 ± 0.94	100
August–September	Chenkan	40	2	31.12 ± 0.97	100
	Punta Xen	0	65	31.47 ± 0.73	100
		0	8	32.41 ± 0.89	100

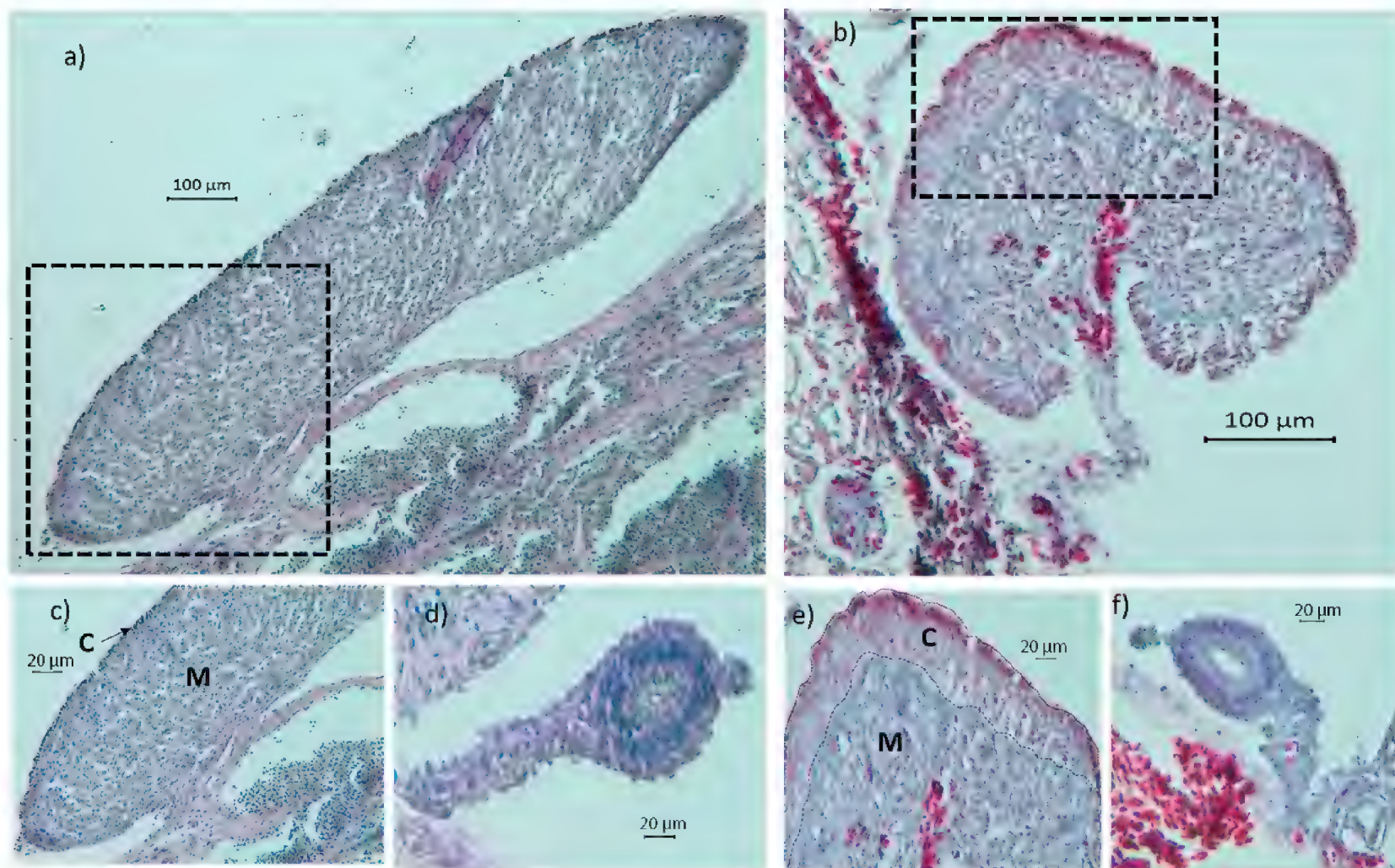


Fig. 4. Representative histology sections of gonads from dead Hawksbill hatchlings in the monitored nests. **(a)** Testis (100 µm), **(b)** Ovary (100 µm), **(c)** Testis (20 µm), **(d)** Müllerian ducts of male (20 µm), **(e)** Ovary (20 µm), **(f)** Müllerian ducts of female (20 µm). The dotted areas in **(a)** and **(b)** indicate the sites of the higher magnifications shown in **(c)** and **(e)**, where the dotted line indicates the cortex, **M** indicates the medulla region, and **C** indicates the cortex region.

as summer houses, restaurants, *palapas*, and fishing areas. In addition, there were breakwaters in the sea. Punta Xen beach had an average width of 22.86 ± 11.55 m. The disturbance represented 14.95% of the length of the beach, mainly in places which were recreation areas, as well as areas with high deforestation rates and the presence of infrastructures, such as a hotel complex. Celestún was the most well-preserved beach, presenting only 6.03% disturbance, mainly due to the presence of two hotel complexes and some summer houses. The average width of the beach was 42.59 ± 6.69 m.

According to the results, the percentage of shade above the nests was the main factor that decreased their temperatures, approaching a PT. Therefore, the percentage of shaded nests, the degree of conservation of the beaches, and the number of nests per km were used to estimate the percentage of nests with temperatures close to the pivotal for the 2017 nesting season. Chenkán beach had the greatest feminization among all the nests, showing only 0.5% of shaded nests. Punta Xen beach had the highest percentage of HV near the nesting areas, so it is estimated that 39.54% of the nests would have temperatures close to the pivotal (shaded nests). For Celestún beach, the HV was far from the nesting areas, so only 21.54% of the nests would have been under the shade.

With respect to the average rainfall, it differed between the two incubation periods on the beaches of Punta Xen and Chenkan. For June–July it was 10.61 ± 4.66 mm, while for August–September it was 7.22 ± 2.68 mm. In Celestún, precipitation was very low during both periods of incubation, without even reaching 1 mm

of precipitation, while the relative humidity remained between 74% and 76% for all incubation months, increasing to 95% in September.

Discussion

In species with genetic sex determination systems, Fisher's theory predicts that the sex ratio in a population tends toward 1:1 (males:females). Given that when either sex is in the majority, the other sex has an advantage in finding mates, an equal sex ratio represents the evolutionarily stable strategy. However, in organisms with temperature-based sex determination this balance can become biased by warm or cold environments (Janzen and Phillips 2006).

The beaches have different environmental temperatures, which provide diverse thermal gradients to the nests (Diffenbaugh and Field 2013; Hawkes et al. 2009; Marcovaldi et al. 2014), so differences in incubation temperatures among nesting beaches would be expected. In the present study, despite the differences in littoral characteristics and conservation status among the three monitored beaches, and the distinct geographical position of Celestún relative to the other two beaches, no significant differences in incubation temperatures were found. This result may be due to the nesting characteristics of the Hawksbill Turtles, since most nests were found in the dune zone, which is associated with vegetation (Horrocks and Scott 1991), and nest temperatures are influenced by the type of vegetation present in each beach. Nevertheless, the low vegetation (LV) and the edge of the high vegetation (HV) do not provide lower

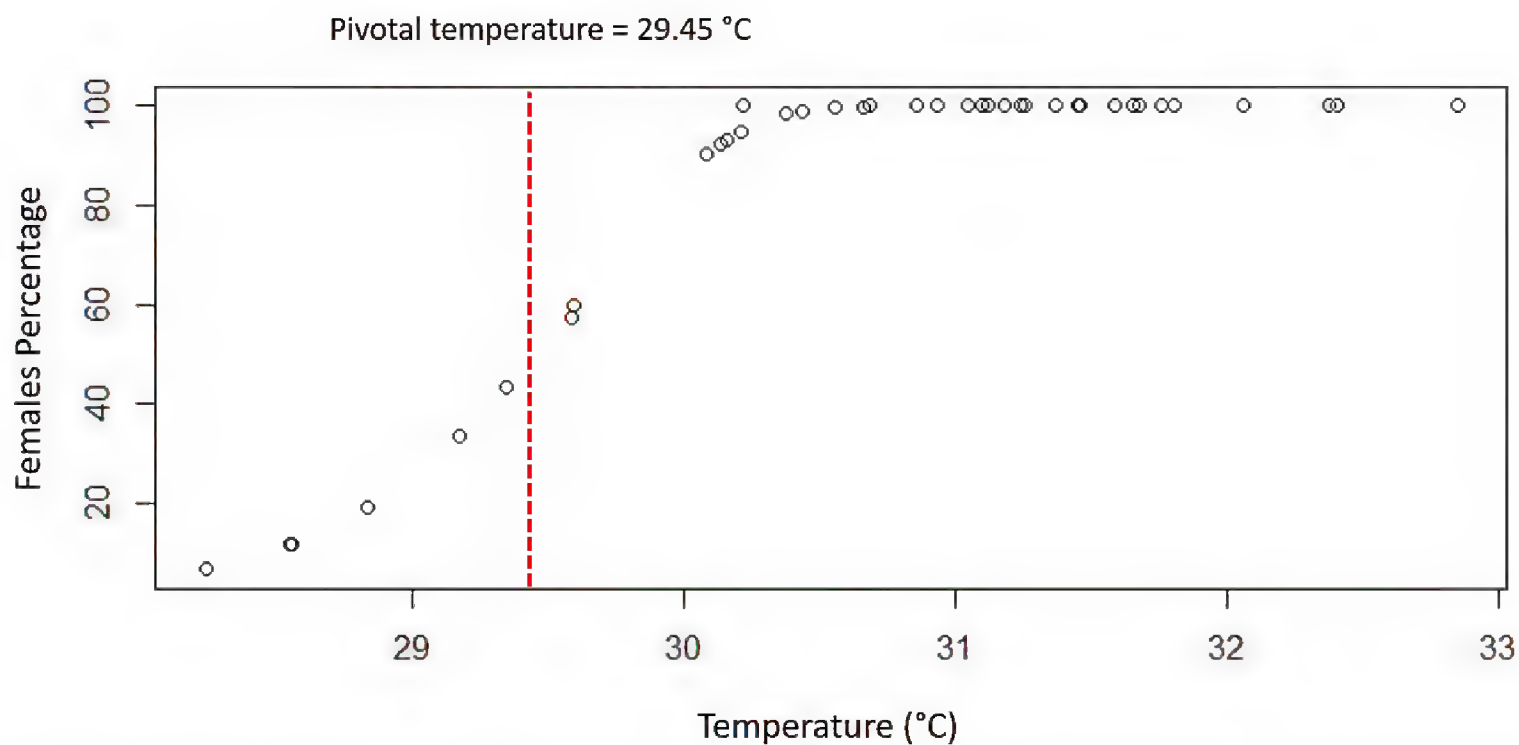


Fig. 5. Relationship between temperature and sex ratio. The red dotted line denotes the pivotal temperature according to the TSD software and calibrated with histology of gonads from dead Hawksbill hatchlings.

temperatures, and only the direct influence of the HV promotes lower temperatures and therefore more male hatchlings (Kamel 2013; Kamel and Mrosovsky 2006). On all three beaches, the vegetation in the nesting areas was low, which failed to decrease the temperatures. Also, the HV near the nests was scarce (less than 30% of the total nests by locality), which caused the majority of the nests to exhibit similar thermal conditions. However, the conditions of each beach differed in the factors that determine the nest temperatures. On Chenkan beach, the least HV occurred because of the high level of anthropogenic impact, primarily by the road that divided the nesting area from the native HV. As a consequence, nesting was prevented in the areas with lower temperatures (below the mangrove), which caused the majority of the nests to be exposed to feminizing temperatures. In contrast, although Celestún was the most well-preserved beach, the HV was generally found inland from the coastal dune, so the distance between the zone of HV and the coastline was wide. On all three beaches, the distance from the nests to the coastline was an average of ~ 17.83 m, and most turtles nested in the dunes. As a consequence, few turtles reached the zone of HV, which was located at an average of ~ 45.59 m from the coastline. Therefore, the majority of turtles nested in areas near LV, so most of the nests would have been in feminizing temperatures. Punta Xen beach had patches with HV (mangroves), so it was the beach with the highest percentage of shaded nests. Thus, the incubation temperatures among the nests were diverse, generating the highest percentage of nests close to the PT among the three monitored beaches. However, in this camp the nests are routinely translocated to hatcheries, due to high predation, so it is not known how this action would affect the sex ratio of the hatchlings. Therefore, despite the differences in the beaches, the trend toward feminization of Hawksbill Turtle hatchlings remained. Unfortunately, in the western Yucatán peninsula, the environmental temperatures are warmer now than 40 years ago (Sinervo

et al. 2010), and they also are in the studied beaches (~ 2.33 °C; CONAGUA). Even though vegetation can play an important role in reducing the nest temperatures, as environmental temperatures continue to rise, the trend toward feminization of hatchlings will increase.

Sea turtles lay several nests over a nesting season, so the environmental temperatures trigger differences in the sex ratio, with a higher percentage of female hatchlings produced in the middle of the nesting period (Mrosovsky et al. 1984). The seasonality in our study area also modified the nest temperatures and, therefore, the sex ratio of the hatchlings. However, contrary to the study of Mrosovsky et al. (1984), we found higher temperatures (~ 31.89 °C) at the end of the nesting season (August–September) than during the middle (June–July, ~ 30.78 °C). These lower temperatures were due to the early rains (June–July) that reduced the nesting temperature and increased the proportion of male hatchlings (Wyneken and Lolavar 2015). In addition, the rain causes an increase in foliage, so most of the nests in June–July were shaded, which influenced the temperature difference between the two incubation periods.

Hatching success did not vary significantly among the beaches and months studied. In contrast, Pike (2008, 2009) previously found that beaches with greater anthropogenic influences have reduced hatching success compared to better preserved beaches. Although we did not observe a change in the percentage of hatching among beaches, the degree of conservation did influence the survival of the nesting females. Particularly at Chenkan beach, several nesting females (six) were run-over by cars while trying to cross the road in order to nest. Nest predation was the main factor that influenced hatching success in the three studied beaches. As this factor was similar on all three beaches, the recruitment of hatchlings of both sexes to the sea did not change. However, on Punta Xen beach it was possible to observe greater predation of the nests left *in situ*, which could be related to the shortest distance between the mangrove and the beach, as well

as that beach's low level of anthropogenic impact, which allowed natural predators of turtle eggs to gain greater access to the nests.

According to the temperatures recorded in all the monitored nests, incubation temperatures did not approach the thermal limits that prevent embryonic development (25–34 °C; Howard et al. 2014). The lowest temperature recorded in the nests was 28.55 °C, while the highest temperature was 32.85 °C, which produced hatching successes of 83.84% and 81.54%, respectively. Therefore, in these locations, the incubation temperatures are still maintained within the optimum temperatures for the embryonic development of Hawksbill Turtles.

Based on gonad histology and the temperatures recorded during the TSP of nests *in situ*, the feminizing temperatures (30.21 °C and above) were determined, as well as the temperature range that produces both sexes (28.55–29.59 °C) for this population. These temperatures are similar to those reported for other populations of Hawksbill Turtles, including those obtained under controlled incubations. This is because the depth of the nests provides a buffer that prevents environmental fluctuations (Booth 2006), and the temperatures of nests *in situ* show minimal variation (± 0.07 °C) during the TSP, remaining close to the temperature variations in experiments under laboratory conditions (± 0.05 °C; Mrosovsky et al. 2009). Therefore, these results suggest that the use of dead hatchlings from nests *in situ* is a reliable way to estimate sex ratios among hatchling sea turtles.

According to controlled experiments in Australian Hawksbill populations, temperatures below 28.4 °C were masculinizing, while temperatures between 28.9 and 29.8 °C produced both sexes, and the temperatures that only produced females were 30.4 °C and above (Loop et al. 1995). In this study, no nests were found with exclusively masculinizing temperatures, since the lowest temperature registered during the TSP was 28.55 °C, from which most of the samples were males (83.33%), but some females were still found (16.67%). Likewise, in 1997, a study in Bahia, Brazil, found the feminizing temperature was 30.4 °C and above (Godfrey et al. 1999). However, in 1998, a study in Milman, Australia, found temperatures of 28 °C and below were masculinizing, whereas between 29.5 and 31 °C both sexes were produced, and at 32.5 °C only females were found (Dobbs et al. 2010). Those studies showed higher pivotal and feminizing temperatures compared to the present study. In general, the temperatures recorded in previous studies were similar to the results here, despite the geographical distances, as well as the differences in time.

The pivotal temperature (PT), determined according to the histological results and the TSD software, was 29.45 °C, which is close to the PT interval of other studies, even for different species, locations, and years (Hawkes et al. 2009). Specifically for *E. imbricata*, in controlled

incubations on Antigua in 1989, the PT was found to be 29.2 °C (Mrosovsky et al. 1992). Similar results were found on Milman Island, Australia, in 1998 (Dobbs et al. 2010); and in Bahia, Brazil, in 1997, the PT was reported as 29.66 °C (Godfrey et al. 1999). Therefore, it appears that the PT has remained relatively constant over the years and in different regions, which supports the hypothesis that this trait is highly conserved in sea turtles (Hawkes et al. 2009; Mrosovsky et al. 1992). This constancy makes it unlikely that the PT in sea turtles will evolve substantially in response to climate change events, which are occurring very rapidly on an evolutionary timescale (Hawkes et al. 2009), and are thus leading to feminization in many populations.

This study highlights the trend towards the feminization of the Hawksbill Turtles, at least in the western Yucatán Peninsula during 2017, since 82.05% of the monitored nests produced only female hatchlings. This trend toward increased female production was present in all monitored beaches, most remarkably in Chenkan. This feminization trend of Hawksbill Turtles has already been reported in previous studies. For example, from 1991 to 1997, in Bahia, Brazil, the sex ratio was estimated according to the incubation duration. Across six nesting seasons, the percentage was greater than 90% females (Godfrey et al. 1999). Wibbels et al. (1999) also observed a strong tendency toward feminization on Buck Island. Based on gonad histology of dead hatchlings in 51 nests *in situ*, 49 nests produced only females, while in the two remaining nests both sexes were found. Likewise, Glen and Mrosovsky (2004) reported changes in the sex ratio of Hawksbill hatchlings in Antigua, relative to those found in the work of Mrosovsky et al. (1992), which found in 1989 that sand temperatures were below the PT. When repeating this earlier work in 2003, Glen and Mrosovsky (2004) reported an increase in air temperature which increased the temperature for most of the nests above the PT. So, they proposed that in the face of climate change, the ratio of males will be reduced. In Bahia and Rio Grande do Norte, Brazil, data from 27 years of incubation durations found that 89–96% of the hatchlings were females in both locations (Marcovaldi et al. 2014).

This trend towards feminization has been documented mainly in hatchlings. However, Hawkes et al. (2013) made a summary of the sex ratios of Hawksbill Turtles and found that the bias toward feminization is greater in hatchlings than in young and adult turtles, suggesting that it may be due to high predation during this stage. Nevertheless, in a study in Anegada, a site characterized by an important foraging aggregation of Hawksbill Turtles from different areas of the Caribbean, Hawkes et al. (2013) measured the levels of testosterone and oestradiol in the blood of juvenile Hawksbill Turtles, and found 2.4- to 7.7-fold more females than males. So, between 69% and 89% of juvenile Hawksbill Turtles were females, which also shows a greater feminization during the juvenile stage. In adults and sub-adults, the sex

ratio of Hawksbill Turtles in Cuba in 1985 and 1986, was 77% females (Carrillo et al. 1998). Therefore, according to the reviewed studies, the feminization trend has been maintained for all life stages of Hawksbill Turtles in the Caribbean.

The Hawksbill Turtle population of the Yucatán Peninsula exhibits high philopatry to the nesting and breeding areas (Cuevas et al. 2012; Labastida-Estrada et al. 2019) among both females and males (González-Garza et al. 2015). The bias towards feminization can thus increase their vulnerability to inbreeding due to decreased gene flow (Hudson 1998). This trend may be exacerbated if the connection among the populations is lost due to habitat loss and fragmentation (Witherington et al. 2011), which could cause a reduction in population sizes, decreasing their genetic variability and increasing the risk of extinction (González-Garza et al. 2015; Spielman et al. 2004).

In the Yucatán Peninsula, two management units (subpopulations) are established in the Hawksbill Turtles, according to their haplotype composition. Genetically, the turtles segregate into two populations: one in the Gulf of Mexico (west of the Yucatán Peninsula) and one in Yucatán and Quintana Roo (northeast of the Yucatán Peninsula; Abreu-Grobois et al. 2003; Labastida-Estrada et al. 2019). González-Garza et al. (2015) found differences in the rates of multiple paternity between these two subpopulations of Hawksbill Turtles, with only the hatchlings from north of the Yucatán Peninsula exhibiting multiple paternity. The lack of multiple paternity among hatchlings from the Gulf of Mexico, may be a consequence of reduced mate availability (Bowen and Karl 2007; Tedeschi et al. 2014).

Hawksbill populations of the Yucatán Peninsula contain endemic haplotypes (Labastida-Estrada et al. 2019), which can be explained by historical patterns of gene flow among populations (Reece et al. 2005). The population of the Yucatán Peninsula is genetically isolated from other populations of the Caribbean, because gene flow decreases across the Caribbean populations towards the Yucatán, due to the emergence of the Campeche bank and the Florida Shelf during the Pleistocene (Reece et al. 2005). At present, in Hawksbill Turtle populations on the Yucatán Peninsula, a lower genetic variability has already been documented in the hatchlings of females that nested for the first time (neophytes) as compared to the hatchlings of remigrant females, which suggests a loss of genetic variation through time (González-Garza et al. 2015). This trend may be exacerbated by the bias toward feminization in this population of Hawksbill Turtles, as revealed in this study.

The bias towards feminization could also be further driven by the effect of climate change, since an increase in temperature even of 1–2 °C, can have considerable effects on the sex ratio (Janzen 1994). In the region of Mexico and Central America, it is estimated that environmental temperatures will exhibit an increase in thermal anomalies of 1.5 to 5 °C, due to the decrease in

precipitation between 2,000 and 2,100 mm (Christensen et al. 2007). Therefore, feminization would increase, limiting the possibility of adaptation and leading to population declines in species that are already in danger of extinction (Hamann et al. 2010; Hawkes et al. 2007; Mitchell and Janzen 2010). In addition, climate change accelerates beach erosion processes, which currently have an estimated erosion rate of 1.8 to 6.8 m/yr for the state of Campeche (Botello et al. 2010). This is driven by human infrastructure, which is the main cause of the destruction of coastal beaches (Botello et al. 2010), as well as the high deforestation that exists in the coastal dunes, severely affecting the nesting and the sex ratio of Hawksbill Turtles, so measures must be taken to reduce it.

Conservation Implications

The trend toward feminization of Hawksbill Turtles has now been documented in several studies, including this one. This bias is predicted to increase in the face of climate change, severely affecting Hawksbill populations, which are already Critically Endangered. Therefore, according to our results, we propose the establishment of conservation strategies that promote balanced sex ratios in these turtles. Since the shade provided by the vegetation is a natural component that decreases the nest temperatures, and therefore maintains the sex ratio of Hawksbill hatchlings, priority should be given to vegetation preservation on the nesting beaches, as well as the realization of reforestation programs, especially of the native vegetation that provides greater coverage to the nests. In addition, vegetation is vital for the survival of a wide variety of other species and for maintaining the stability and integrity of the beach. In some turtle camps in Mexico, as is the case of Punta Xen, the conservation strategy is to move the nests *in situ* to hatcheries, to reduce their losses due to predation. Therefore, we recommend keeping nests *in situ*, in areas with greater vegetation cover, and carrying out continuous monitoring to reduce the predation. Or, where appropriate, we recommend the use of at least 50% artificial shade in the hatcheries, in order to provide more equitable recruitment of hatchlings in terms of their sex ratio. These measures should continue to promote the recovery of Hawksbill Turtle populations, and ensure their viability in the face of climate change.

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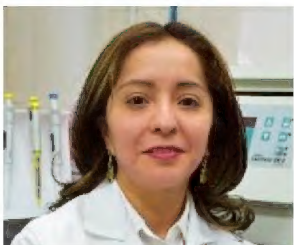
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Cynthia Dinorah Flores-Aguirre is a Ph.D. candidate in the postgraduate program in Biological Sciences at the Universidad Nacional Autónoma de México (UNAM). Her main interests are the conservation, ecology, and behavior of various fauna, as well as their vulnerability to climate change, focusing on the Mexican herpetofauna.



Verónica Díaz-Hernández is a Biologist who received her Ph.D. in Biomedical Sciences from the Universidad Nacional Autónoma de México (UNAM) in 2008. Verónica is currently a Full Professor in the Department of Embryology at UNAM. Her main interests are in sexual determination among reptiles, and more recently in the vulnerability of reptiles to climate change.



Isaías Hazarmabeth Salgado Ugarte is a senior Full Professor at Laboratorio de Biometría y Biología Pesquera, Facultad de Estudios Superiores Zaragoza Campus, Universidad Nacional Autónoma de México (UNAM). Isaías earned his Bachelor of Science in Biology, and his Master of Science in Biological Sciences from UNAM; and he obtained a Fisheries Biology Specialty and a Ph.D. in Aquatic Biosciences (Fisheries Biology) from University of Tokyo, Japan. He has been working on exploratory, confirmatory, univariate, bivariate, multivariate, computer intensive statistical methods (nonparametric kernel density and regression smoothing, cross-validation, bootstrapping) to analyze biological data.



Luis Enrique Sosa Caballero is a Biologist who graduated from the Facultad de Ciencias, Universidad Nacional Autónoma de México in 2015. Luis completed his Master of Science in the Institute of Biology of the same university, obtaining the degree with honorable mention in 2019. His main topics of interest are conservation, climate change, and thermoregulation, focusing on groups of amphibians and reptiles, with special attention on reptile species which have sex-determination by temperature, such as sea turtles and crocodiles. In addition, he has an interest in the geographical distributions of different groups under predictive scenarios and climate change.



Fausto R. Méndez de la Cruz is a Full Professor at the Universidad Nacional Autónoma de México (UNAM) in México City. Fausto obtained his Ph.D. in 1989 from UNAM and did postdoctoral studies at the University of Florida, Gainesville, Florida, USA, and a sabbatical at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA. His research is focused on the evolution of reproductive systems in reptiles, mainly on the evolution of viviparity and parthenogenesis, and more recently on the vulnerability of amphibians and reptiles to climate change.